

# **UNIVERSITI PUTRA MALAYSIA**

CHARACTERIZATION, PATHOGENICITY AND HOST RANGE ANALYSES OF Lasiodiplodia sp. AND RELATED SPECIES ISOLATED FROM FRUIT ROT DISEASE OF MANGO IN PENINSULAR MALAYSIA

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FS 2017 60



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MUNIRAH BINTI MOHD SATTAR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia. In Fulfillment of the Requirements for the Degree of Master of Science

April 2017

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

## CHARACTERIZATION, PATHOGENICITY AND HOST RANGE ANALYSES OF *Lasiodiplodia* sp. AND RELATED SPECIES ISOLATED FROM FRUIT ROT DISEASE OF MANGO IN PENINSULAR MALAYSIA

By

#### **MUNIRAH BINTI MOHD SATTAR**

April 2017

Chairman: Nur Ain Izzati Mohd Zainudin, PhD Faculty: Science

Lasiodiplodia species is an important plant pathogen causing fruit rot, a threatening disease for mango fruits in Malaysia. Capability of thespecies to infect during preand post-harvest phases will reduced mango production and creating tremendous losses in country economic. The information regarding pre-harvest fruit rot disease on mango in Malaysia is still lacking, therefore, this study was conducted. During a series of sampling in July 2014 to May 2015 throughout Peninsular Malaysia, the symptom of fruit rot was observed in the Malaysian mango recommended varieties such as Chok Anan (MA224), Harumanis (MA128), Sala and Epel. Thus, the objectives of this study were to isolate and identify fungi species isolated from mango fruit rot disease based on morphological and molecular approaches using internal transcribed spacer (ITS) and  $\beta$ -tubulin gene (BT2) sequence, to determine whether or not Lasiodiplodia, Pseudofusicoccum and Neofusicoccum isolates are pathogenic and to examine the host range of Lasiodiplodia theobromae, Lasiodiplodia pseudotheobromae and Pseudofusicoccum adansoniae as causal pathogens of mango fruit rot disease in selected commercial fruits. There are 26 isolates were purified and successfully identified as Lasiodiplodia theobromae (17 isolates), Lasiodiplodia pseudotheobromae (3 isolates), Pseudofusicoccum adansoniae (5 isolates) and Neofusicoccum parvum (1 isolate). A combined phylogenetic tree was constructed using maximum-likelihood method showed the same species grouped into the same clade. Clade I consisted of L. theobromae, L. pseudotheobromae and N. parvum while Clade II were P. adansoniae species. For the pathogenicity test, all isolates of Lasiodiplodia species inoculated on healthy mango fruit using non-wounded method are pathogenic and showed fruit rot symptom except for L. pseudotheobromae (B1494). Whereas, for P. adansoniae and N. parvum species isolates, only one isolate of P. adansoniae (B1474) produced fruit rot symptom. The most virulent isolate was L. theobromae (A1718) with disease severity index, DSI of 87.50%. To examine virulence of the pathogenic isolates obtained from mango samples on different hosts, each species isolates with highest



DSI in pathogenicity test were inoculated on seven commercial fruits such as apple, banana, musk lime, guava, mandarin, pear and sapodilla. The host range study confirmed that *L. theobromae, L. pseudotheobromae and P. adansoniae* were pathogenic to apple, musk lime, guava, mandarin and sapodilla with varies in disease severity. As conclusion, *L. theobromae, L. pseudotheobromae* and *P. adansoniae* were identified and confirmed as pathogens of pre-harvest fruit rots disease on mango in Malaysia. Those three species are also potential pathogen for apple, banana, musk lime, guava, mandarin and sapodilla if dissemination of inoculums were occurred during pre- or post-harvest stages. This study represents the first report of *L. pseudotheobromae* and *P. adansoniae* on pre-harvest mango in Peninsular Malaysia associated with fruit rot disease.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

## ANALISA PERINCIAN, UJIAN KEPATOGENAN DAN KEPELBAGAIAN PERUMAH *Lasiodiplodia* sp. DAN SPESIES BERKAITAN YANG DIPENCILKAN DARI PENYAKIT REPUT BUAT MANGGA DI SEMENANJUNG MALAYSIA

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Spesies Lasiodiplodia adalah pathogen tumbuhan yang penting penyebab reput buah, penyakit pengancam bagi buah mangga di Malaysia. Keupayaan spesies ini menyebabkan penyakit reput buah semasa peringkat pra- dan lepas-tuai telah menurunkan pengeluaran mangga dan memberi kesan kepada ekonomi negara. Oleh kerana informasi berkaitan penyakit reput buah mangga di Malaysia pada peringkat pra-tuai masih tiada, oleh itu kajian ini dijalankan. Semasa persampelan dijalankan pada Julai 2014 hingga Mei 2015 di seluruh Semenanjung Malaysia. Simptom penyakit reput buah telah diperhatikan pada varieti mangga Malaysia terpilih iaitu Chok Anan (MA224), Harumanis (MA128), Sala dan Epel. Oleh itu, objektif bagi kajian ini adalah untuk memencilkan dan mengenalpasti spesis kulat dari buah mangga yang mengalami reput buah berdasarkan pendekatan morfologi dan molekular menggunakan jujukan transcribed spacer (ITS) dan gen  $\beta$ -tubulin (BT2), untuk mengkaji sama ada pencilan Lasiodiplodia, Pseudofusicoccum dan Neofusicoccum adalah patogenik dan untuk memeriksa pelbagai perumah bagi spesies theobromae, Lasiodiplodia pseudotheobromae Lasiodiplodia dan Pseudofusicoccum adansoniaeyang merupakan penyebab reput buah mangga pada buah komersial yang terpilih. Terdapat dua puluh enam pencilan kulat dipurifikasi dan berjaya dikenalpasti iaitu Lasiodiplodia theobromae (17isolat), Lasiodiplodia pseudotheobromae (3 isolat), Pseudofusicoccum stromaticum (5 isolat) dan Neofusicoccum parvum (1 isolat). Gabunganpohon filogenetik dibina berasaskan teknikmaximum-likelihood menunjukkan semua spesies yang sama dikumpulkan dalam clad yang sama. Clad I terdiri daripada spesies L. theobromae, L. pseudotheobromae dan N. parvum, manakala Clad II ialah spesis P. adansoniae. Untuk ujian kepatogenan, semua isolat spesies Lasiodiplodiayang diinokulasi ke atas buah manga yang sihat menggunakan teknik tanpa torehan adalah patogenik dan menunjukkan simptom reput buah kecuali L. pseudotheobromae (B1494). Dimana, untuk isolat spesies Pseudofusicoccumdan Neofusicoccum, hanya satu isolat P. adansoniae (B1474) menghasilkan simptom reput buah. Isolat yang paling virulen



adalah *L. theobromae* (A1718) dengan DSI87.50%. Untuk memeriksa tahap kevirulenan spesies patogenik pelbagai perumah, kulat-kulat ini diuji ke atas tujuh buah komersial seperti epal, pisang, limau kasturi, jambu batu, limau, pir dan ciku. Kajian ke atas kepelbagaian perumah membuktikan *L. theobromae, L. pseudotheobromae* dan *P. adansoniae*patogenik ke atas epal, limau kasturi, jambu batu, limau dan kendondong dengan pelbagai tahap keseriusan penyakit. Kesimpulannnya, *L. theobromae, L. pseudotheobromae* dan *P. adansoniae* telah diidentifikasi dan disahkan sebagai pathogen penyakit reput pra-tuaibuah manga di Malaysia. Kesemua tiga spesies berpotensi sebagai patogen epal, pisang, limau kasturi, jambu batu, limau dan ciku jika pendedahan inokulasi berlaku semasa peringkat pra- dan lepas-tuai.Kajian ini merupakan laporan pertama berkenaan *L. pseudotheobromae* dan *P. adansoniae* dan *P. adansoniae* dan *P. adansoniae* dan *B. adansoniae* dan *A. pseudotheobromae A. adansoniae A. pseudotheobromae A. pseudotheobromae A. adansoniae A. pseudotheobromae A. pseudotheobromae A. pseudotheobromae A. pseudotheobromae A. adansoniae A. pseudotheobromae A. adansoniae A. pseudotheobromae A. adansoniae A. pseudotheobromae A. pseudotheobromae A. adansoniae A. pseudotheobromae A. adansoniae A. adansoniae A. pseudotheobromae A. pseudotheobromae A. adansoniae A. pseudotheobromae A. adansoniae A. pseudotheobromae A. pseudotheobromae A. adansoniae A. pseudotheobromae A. pseudotheobromae A. adansoniae A. pseudotheobromae A. pseudotheobr* 



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# LIST OF ABBREVIATIONS

ASEAN	Association of Southeast Asian Nations
BLAST	Basic Local Alignment Search Tool
bp	Base pair
BT2	β-tubulin gene
CMX	Complete Medium Xylose
DNA	Deoxyribonucleic Acid
DOA	Department of Agriculture
DSI	Disease Severity Index
EF1-α	Elongation Factor 1-alpha
ETS	External transcribed spacer
FAO	Food and Agriculture Organization
FASTA	Fast Alignment Search Tool
ha	Hactare
IGS	Intergenic spacer
ITS	Internal Transcribed Spacer
ML	Maximum Likelihood
Mt	Metric tone
NCBI	National Centre for Biotechnology Information
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SNA	Synthetic low-nutrient agar
SSU	Small subunit
TBE	Tris Borate Acid
USD	United States Dollar
UV	Ultraviolet
WA	Water Agar

### **CHAPTER 1**

#### INTRODUCTION

Plant diseases have caused tremendous losses of crop throughout the world. They can attack the plants during pre- and postharvest stages. The losses from plant diseases may produce catastrophic results when the crops are highly depends as a food sources. Previously, the history has shown, outbreak of crop disease has caused starvation, death and mass migration of the human population (Nowicki, Foolad, Nowakowska & Kozik, 2012). Therefore, the emphasization regarding the plant diseases knowledge is important to avoid repetition of history.

Generally, a plant becomes diseased due to susceptibility of host plant and any disturbance brought by pathogen or environmental factor that results changes in appearance, physiological process and decrease in yields compared to normal and healthy plant (Agrios, 2005). Between these three factors, diseases caused by pathogenic agents such as fungus, bacteria, virus, viroid and nematode are classified as the infectious disease and more severe because the causal agent is an organism or non-organisms that capable to reproduce and spread from one susceptible host to another with or without aid of vector. In addition, some of the pathogenic agents contribute to the pre- and postharvest disease on crop which both stages have their own significant impact on crop losses (Isaac, Amaglo, Kodzo Kumah & Ofori, 2015). Pre-harvest diseases are spoilages that developed before the process of harvesting, whereas postharvest diseases are deterioration that occurred on crop during harvesting until consumption including handling, packaging, storing, transporting and marketing that lead to completely unmarketable. Although preharvest diseases on fruits are not widely known to give severe loss as postharvest, but wrong practices in handling the disease may seriously affect the postharvest quality. Besides, a good quality of fruit comparatively depended on certain practices that have been carried out during pre-harvest fruits production (Isaac, Amaglo, Kodzo Kumah & Ofori, 2015).

There are various vegetables and fruits crops grown in Malaysia are susceptible with one or more range of diseases. However, this study was focused on the preharvest disease of mango associated with fruit rot. Mango is scientifically known as *Mangifera indica* in botanical taxonomic is chosen in this study due to widely planted and consumed among Malaysian people as well as globally (Norton, 2016). The demand on mango fruit in Malaysia was increasing (The Report: Malaysia, 2010) such an example, Perlis iconic Harumanis mango. Improving mango production is important in Malaysia since the demand for fresh tropical fruits was estimated increase to 2.7 million mt in 2010 to 3.4 million mt in 2020 (MOA, 2011). As been listed among 12 main fruits in Malaysia Agro-Food Policy (2010-2020), production of mango were enhanced to be utilized as a food source and commercial product in several ways either in cooked form such as pickles, beverages, puree, chocolate and dessert or eaten raw. Moreover, the nutrients content in mango have been revealed to have higher vitamin C compared to several other citrus fruit (Awa,

Samuel, Oworu & Sosanya, 2012) and thus important to include in the diet for improving human health. Besides, in terms of economically importance, difference in fruiting season between Malaysia and other mango exporter countries could provide new opportunity for Malaysia to increase mango exportation (Rosidah, Faridah, Jamaliah & Norzaidi, 2010) by given fully attentions to control the plant diseases that lead to decreasing of mango production.

Mango is exposed to wide range of pathogen and diseases (Tharanathan, Yashoda & Prabha, 2007) at every stages of their life. Among them, diseases caused by fungal infection such as anthracnose, stem end rot and mango decline are the most destructive and the losses can reach up to 100% (Barkai-Golan, 2001; Galdino et al., 2016). In this study, the attention has been given to fruit rot disease that commonly observed in mango orchard throughout Peninsular Malaysia showing the initial symptom of black spots with brown colour of edges and have distinct in shape. Later on, the symptom experienced pulp exposing as the ripening process increased. The fruit rot disease is caused by Lasiodiplodia theobromae from family of Botryosphaeriaceae that causing infection at both field and postharvest stages. This species which consists of species complex when identified using multiple gene sequence (Marques, Lima, Antônio & Jr, 2013) has reportedly produces similar symptom in many other economic crops such as crown rot disease on banana, fruit rot of coconut and stem end rot in mango (Khanzada, Lodhi & Shahzad, 2005). In Malaysia, there is no previous study of L. theobromae associated with fruit rot disease on mango have been reported eventhough the symptoms were frequently observed. Therefore, this current study will provide the information regarding the pre-harvestdisease of mango associated with fruit rot disease in Malaysia.

Information for this specific plant diseases together with comprehensive understanding of respective fungi plays a vital role in developing plant disease control. Plant disease management is constructed with the goal to reduce the economic and yield loss besides aesthetic damage caused by plant disease. Therefore, accurate identification of causal agent were required by further identified using molecular analysis and pathogenicity test as well as host range analyses to strengthen the result and providing the knowledge of potential host among the commercial fruit of the pathogens.

Previously, identification of fungal disease is focused on morphological characteristics, which include cultural and micro-morphological characters such as conidial features. However, problem arises when a fungal culture undergoes morphological variation, phenotype among species and loses of particular characteristic in different environmental factors that lead to inaccurately identification. For that reason, in this study, the molecular approaches using two genes sequences analyses which are Internal transcribed spacer (ITS) region and  $\beta$ -tubulin gene (*BT2*) were used as universal and specific regionsrespectively for fungi identification among fungal that contained species complex (Abdollahzadeh, Javadi, Mohammadi, Zare & Phillips, 2010). Therefore, gathering appropriate information on the morphological and molecular identify, pathogenicity test and host

range studies for fungal group of Botryosphaeriaceae especially *Lasiodiplodia* species will contribute a better-integrated disease management and improve mango production in country.

The objectives of the study were:

- i. to isolate and identify fungi pathogens isolated from mango fruit rot disease based on morphological and molecular approaches using Internal Transcribed Spacer (ITS) and  $\beta$ -tubulin gene (*BT2*) sequence analysis,
- ii. to determine pathogenicity of *Lasiodiplodia*, *Pseudofusicoccum* and *Neofusicoccum* isolates obtained from the infected mango, and
- iii. to examine the host range of *Lasiodiplodia theobromae*, *L. pseudotheobromae* and *Pseudofusicoccum adansoniae*, causal pathogens of fruit rot disease of mango in selected commercial fruits.



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