



UNIVERSITI PUTRA MALAYSIA

***PHENOTYPIC AND MOLECULAR IDENTIFICATION OF PANTOEA
STEWARTII SUBSPECIES INDOLOGENES OF MANGO (MANGIFERA
INDICA L.) IN SELANGOR***

AHMAD MUSTAQIM BIN AHMAD KHASHIRI

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BERILMU BERBAKTI

**PHENOTYPIC AND MOLECULAR IDENTIFICATION OF *Pantoea stewartii*
subspecies *indologenes* OF MANGO (*Mangifera indica* L.) IN SELANGOR**

By

AHMAD MUSTAQIM BIN AHMAD KHASHIRI

**Thesis Submitted to the Department of Plant Protection, Faculty of Agriculture,
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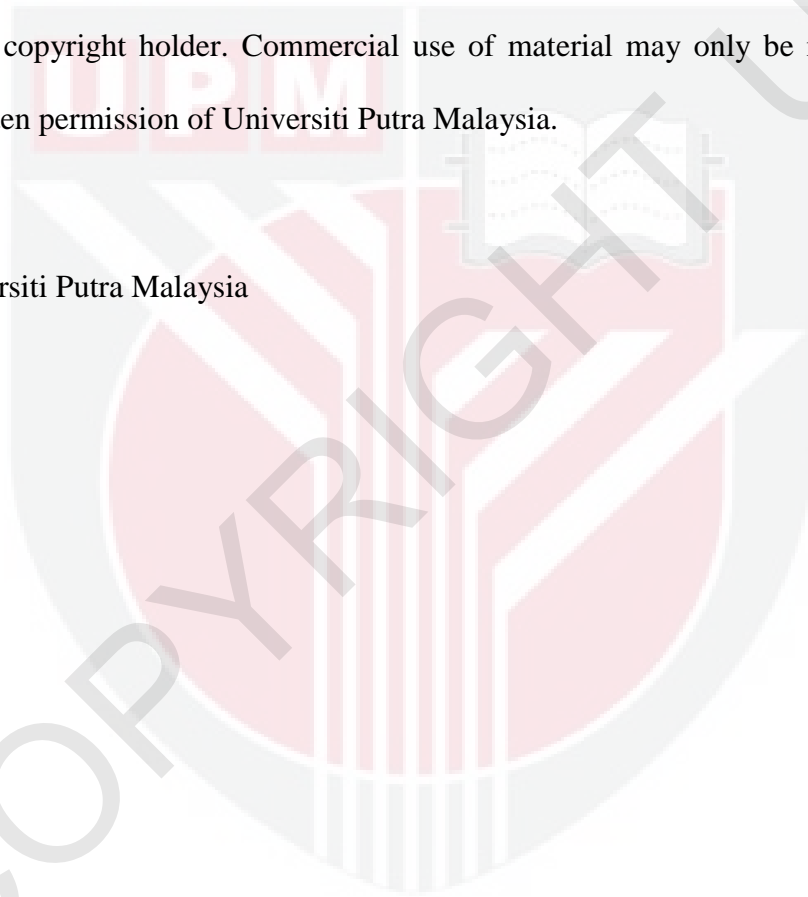
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Abstract of the thesis represented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the Bachelor`s Degree

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ABSTRACT

Mango (*Mangifera indica* L.) is among the most cultivated fruits in Malaysia, especially in the Northern regions with low rainfall rates. This fruit is produced for domestic and international market. However, a bacterium known as, *Pantoea stewartii* subspecies (spp.) *indologenes* can cause diseases in mango that are undetected until now. In general, the unknown diseases does not cause extreme losses, but in under favourable condition it can cause damages. Furthermore, symptoms showed by *Pantoea stewartii* spp. *indologenes* are similar as the bacterial black spot disease such as black scabby spots on the fruits. The objectives of this study were 1) to isolate and identify *Pantoea* spp. in local mango cultivars via phenotypic characterization, and 2) to determine the relationships of *Pantoea stewartii* spp. *indologenes* strains isolated in this study via molecular

approaches. To achieve these objectives, infected mango fruits with bacterial black spot symptoms were obtained from Taman Pertanian Universiti UPM, Puchong, Selangor. In phenotypic characterization, the morphology of the bacterial strains isolated from the infected mango fruits were identified as yellow, slightly mucoid, mounded and circular in shape. Gram-staining revealed that all strains were Gram-negative with short rod-shaped. Pathogenicity test performed on mango seedlings of 'Chok Anan' variety with all strains induced symptoms that were damage on the upper side of the leaves and center of the leaves turned grey surrounded by a yellow halo. The PCR amplification of 16S rDNA region in all isolated strains produced an amplicon of ~1400 basepair (bp) in size. Sequencing analysis revealed that all strains were 99% identical to *Pantoea stewartii* spp. *indologenes* reference strains in GenBank database (Accession nos. HG792424 and HG792422). Phylogenetic analyses of 16S rDNA gene sequences further clustered all strains into *Pantoea stewartii* spp. *indologenes* reference sequences strains. To our knowledge, this is the first report of mango disease caused by *Pantoea stewartii* spp. *indologenes* in Malaysia.

Abstrak tesis ini dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Muda

**Pengenalan Fenotip dan Molekul *Pantoea stewartii* subspesies *indologenes*
pada Mangga (*Mangifera indica* L.) di Selangor**

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ABSTRAK

Mangga (*Mangifera indica* L.) adalah diantara buah-buahan yang kerap ditanam di Malaysia, terutamanya di kawasan Utara dengan kadar hujan yang rendah. Buah ini dihasilkan untuk pasaran domestik dan antarabangsa. Walaubagaimanapun, bakteria yang dikenali sebagai *Pantoea stewartii* subspesies (spp.) *indologenes* boleh menyebabkan penyakit pada mangga yang tidak dapat dikesan sehingga sekarang. Secara umum, penyakit yang tidak diketahui tidak menyebabkan kerugian melampau, tetapi di bawah keadaan yang menggalakkan ia boleh menyebabkan kerosakan. Tambahan pula, gejala yang ditunjukkan oleh *Pantoea stewartii* spp. *indologenes* adalah sama seperti penyakit bintik hitam bakteria seperti bintik-bintik kuring hitam di bahagian buah-buahan. Objektif kajian ini adalah 1) untuk mengasingkan dan mengenal pasti *Pantoea* spp. dalam kultivar mangga tempatan melalui pencirian fenotip, dan 2) untuk menentukan hubungan *Pantoea stewartii* spp. *indologenes* strain diasingkan dalam kajian ini melalui pendekatan molekul.

Untuk mencapai objektif ini, buah-buahan mangga dijangkiti gejala tempat hitam bakteria diperolehi dari Taman Pertanian Universiti UPM, Puchong, Selangor. Dalam pencirian fenotip, morfologi strain bakteria diasingkan daripada buah mangga yang dijangkiti telah dikenal pasti sebagai kuning, sedikit berlendir, sedikit berbukit dan dalam bentuk bulat. 'Gram-staining' mendedahkan bahawa semua strain adalah Gram-negatif dengan berbentuk batang-pendek. Ujian kepatogenik dilakukan pada benih mangga varieti 'Chok Anan' dengan semua jenis gejala-gejala yang menyebabkan kerosakan di sebelah atas daun dan pusat daun bertukar kelabu dikelilingi oleh halo kuning. Amplifikasi PCR pada 16S rDNA dalam semua strain diasingkan dan menghasilkan amplicon ~1400 basepair (bp) dalam saiz. Analisis penjujukan mendedahkan bahawa semua strain adalah 99% sama dengan *Pantoea stewartii* spp. *indologenes* strain rujukan dalam pangkalan data GenBank (No. kesertaan HG792424 dan HG792422). Urutan gen lanjut analisis filogenetik 16S rDNA, berkelompok kepada semua strain *Pantoea stewartii* spp. *indologenes* merujuk urutan strain rujukan. Untuk pengetahuan kita, ini adalah laporan pertama penyakit mangga yang disebabkan oleh *Pantoea stewartii* spp. *indologenes* di Malaysia.

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENT	v
DECLARATION	vi
LIST OF FIGURES AND TABLES	xi
LIST OF ABBREVIATIONS	xii
CHAPTER	
1 INTRODUCTION	1
1.1 Background of the Study	1
1.2 Statement of the Problem	1
1.3 Significance of the Study	2
1.4 Objective of the Study	2
2 LITERATURE REVIEW	3
2.1 History and Global Production of Mango	3
2.2 Mango Cultivation in Malaysia	3
2.3 Bacterial Disease of Mango	6
2.4 <i>Pantoea</i> spp.	7
2.4.1 Distribution and Epidemiology of <i>Pantoea</i> spp.	7
2.4.2 Symptoms Caused by <i>Pantoea</i> spp. in Mango	9
2.4.3 Phenotypic Characterization	12
3 ISOLATION, IDENTIFICATION AND PHENOTYPIC CHARACTERISTIC OF <i>Pantoea</i> spp. FROM MANGO (<i>Mangifera indica</i> L.) IN SELANGOR	13
3.1 Introduction	13
3.2 Materials and Methods	14
3.2.1 Samples collection	14
3.2.2 Isolation of Pure <i>Pantoea</i> spp. from Mango	15
3.2.3 Biochemical Characterization of <i>Pantoea</i> spp.	15
3.2.4 Pathogenicity Test of <i>Pantoea</i> spp. on Mango Cultivars	16
3.3 Results	17
3.3.1 Field Symptoms of <i>Pantoea</i> spp. Infecting	

	Mango	17
3.3.2	Isolation and Morphological Characterization of <i>Pantoea</i> spp. from Mango	19
3.3.3	Biochemical Characterization of <i>Pantoea</i> spp. Oxidase Catalase	20
3.3.4	Pathogenicity of <i>Pantoea</i> spp. in Mango Cultivar	22
3.4	Discussion	25
4	MOLECULAR CHARACTERIZATION AND PHYLOGENY OF <i>Pantoea stewartii</i> spp. <i>indologenes</i> IN SELANGOR	26
4.1	Introduction	26
4.2	Materials and Methods	26
4.2.1	Samples Collection	26
4.2.2	Bacterial DNA Extraction	26
4.2.3	Species-specific PCR Amplification of <i>Pantoea stewartii</i> spp. <i>indologenes</i> Strains	27
4.2.4	Detection of PCR Products	28
4.2.5	DNA Sequencing and Sequence Alignment	28
4.2.6	Phylogenetics Analyses	29
4.3	Results	29
4.3.1	Species-specific PCR Amplification of <i>Pantoea stewartii</i> spp. <i>indologenes</i> Strains	29
4.3.2	Phylogenetic Analyses of <i>Pantoea stewartii</i> spp. <i>indologenes</i> Strains	30
4.4	Discussion	33
5	CONCLUSION	34
	REFERENCES	37
	APPENDICES	46
	BIODATA OF STUDENT	48

LIST OF FIGURES/ TABLES

Figure/ Tables	Page
2.1 Industrial trend of mango cultivation in Malaysia	4
2.2 List of mango variety registered on national list	5
2.3 Major mango disease and their causal pathogens	6
2.4 Symptoms of <i>Pantoea</i> spp. on mango	10
3.1 Sampling area in Puchong, Selangor	14
3.2 Samples collected from different sources and mango varieties in Taman Pertanian Universiti UPM, Puchong.	14
3.3 Field symptoms on Taman Pertanian Universiti UPM in Puchong	18
3.4 Colony morphology of <i>Pantoea</i> spp.	19
3.5 Gram-staining of all strains showed Gram-negative with short rod-shaped bacteria.	21
3.6 Symptoms on leaves of mango Chok Anan seedlings after two (2) weeks inoculation of <i>Pantoea</i> spp.	23
3.7 Symptoms on leaves of mango Chok Anan seedlings after two (2) weeks inoculation of <i>Pantoea</i> spp.	24
4.1 List of samples collected from different sources and mango varieties in Taman Pertanian Universiti UPM, Puchong	26
4.2 The 16S rDNA PCR amplification of total genomic DNA from <i>Pantoea stewartii</i> spp. <i>indologenes</i> strains.	30
4.3 Phylogenetic tree constructed from a Bayesian analysis of the partial 16S rDNA region gene sequences using MrBayes	31
4.4 Origin and characterization of <i>Pantoea stewartii</i> spp. <i>indologenes</i>	32

LIST OF ABBREVIATIONS

%	percent
°C	degree celcius
bp	base pair
DNA	deoxyribonucleic acid
DOA	Department of Agriculture
EDTA	ethylene-diamine-tetraacetic acid
g	gram
h	hour
L	liter
min	minutes
ml	milliliter
PCR	polymerase chain reaction
rpm	rotation per minute
sec	seconds
TAE	tris-acetic EDTA
<i>Taq</i>	<i>Thermus aquaticus</i>
μl	microliter
lbs.	pound

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Mango (*Mangifera indica* L.) is sixth among eleven fruits that was included in Principle of Malaysia Agro Food (2011-2020). In 2011, mango is cultivated at almost 5 million hectares worldwide. Based on, Muda Agriculture Development Authority (MADA) approximately 9,500 hectares area in Malaysia were cultivated with mangoes with the production of more than 25,000 metric tons from different varieties (MADA, 2011). This data showed that mango is among the most important fruit crops that contributes to Malaysia's locally and internationally economic growth.

1.2 Statement of the Problem

Bacterial infections are major constraint for several crops, where they consequential killing of the host resulted in losses that could reach over 50% of the infections (Janse, 2012). *Pantoea* spp. can be one of the causal agent in local mango (*Mangifera indica* L.) cultivar that can cause disease. This aerobic microorganisms were aerobic, Gram-negative and frequently encountered in subtropical and tropical regions (Kageyama *et al.*, 1992).

1.3 Significance of the Study

As mango is among the most important fruits in Malaysia for domestic and international markets, it is important to update the current status of *Pantoea* spp. infection in mango. The results of our study will be an important documentation of *Pantoea* spp., in particularly *Pantoea stewartii* spp. *indologenes* infection in mango cultivars in Malaysia via phenotypic and molecular approaches.

1.4 Objective of the Study

This study was carried out with the following objectives:

1. To isolate and identify *Pantoea* spp. in local mango cultivars via phenotypic characteristics.
2. To characterize the *Pantoea stewartii* spp. *indologenes* strains isolated in this study via molecular identification.

The output from this study will release an updated information of *Pantoea stewartii* spp. *indologenes* isolated from mango in Malaysia. In addition, it may increase the efficiency of an accurate molecular diagnosis test for detection and identification of this bacterium in infected mango.

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