



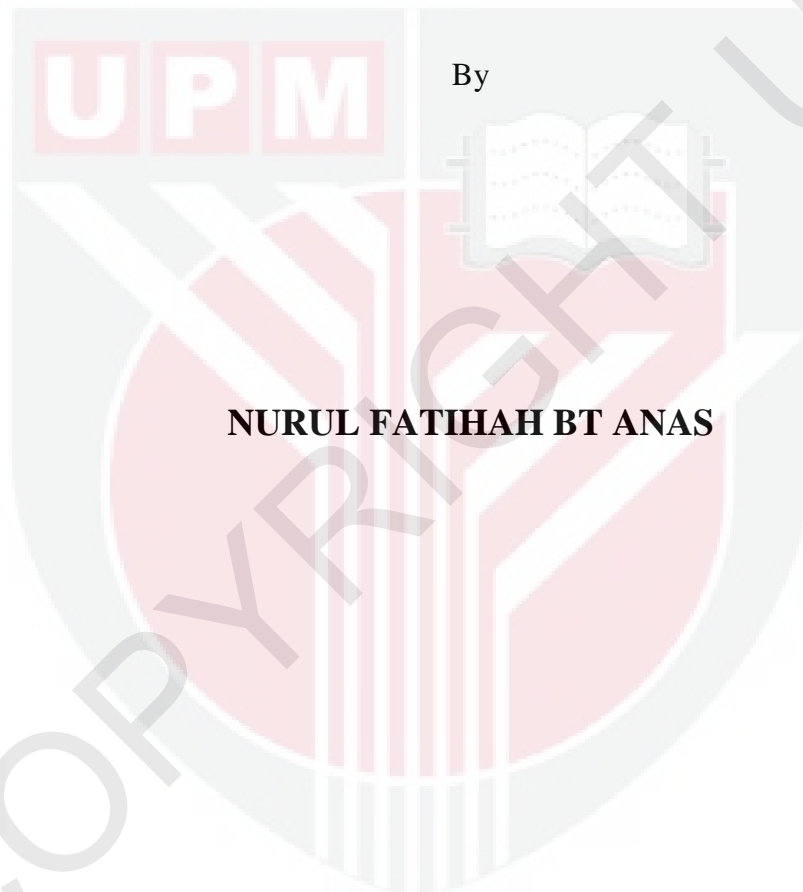
UNIVERSITI PUTRA MALAYSIA

**LACTIC ACID BACTERIA AS BIOLOGICAL CONTROL AGENT AGAINST
PAPAYA DIEBACK DISEASE**

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FBSB 2015 165

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PAPAYA DIEBACK DISEASE**



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Thesis Submitted to the Department of Cell and Molecular Biology, Faculty of
Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, in Fulfillment of
the Requirements for the Degree of Science (Hons.) Cell and Molecular Biology

June 2015

Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfillment of the requirement for the degree of Bachelor of Science (Hons.) Cell and Molecular Biology

LACTIC ACID BACTERIA AS BIOLOGICAL CONTROL AGENT AGAINST PAPAYA DIEBACK DISEASE

By

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June 2015

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Papaya (*Carica papaya*) is an economically fruit crop grown in Malaysia. However, the outbreak of Papaya Dieback disease on papaya industry has caused a rapid declination in papaya production. The aim of the study was to isolate and identify endophytic Lactic Acid Bacteria (LAB) from papaya that able to inhibit *Erwinia mallotivora*, the causative agent of Papaya Dieback disease. The bacterial cultures from papaya seed extract were serially diluted and plated on M17 agar supplemented with 0.5 % glucose. 18 out of 70 colonies were tested positive to inhibit this pathogen using agar disc diffusion assay. The antibacterial activity was recorded by measuring the diameter in (mm) of clear inhibition zone surrounding the disc. The isolate A28 showed the highest clear inhibition zone which is 10 mm. The morphological characterization and biochemical test of the isolated strains indicated that they were Gram-positive, cocci shape and catalase-negative. Molecular characterization of the bacterial endophytes using 16S rRNA sequencing is still in progress. The phylogenetic analysis of 16S rRNA gene sequences will be performed. The selected microbial isolates obtained in this study will be used in controlling Papaya Dieback disease. To our knowledge, the biological approach using LAB to control this disease has not yet been applied in Malaysia.

Keywords: Lactic Acid Bacteria (LAB), *Carica papaya*, Papaya Dieback disease, *Erwinia mallotivora*

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul Sebagai memenuhi keperluan untuk ijazah Sarjana Muda Sains (Kepujian) Biologi Sel dan Molekul

**BACTERIA ASID LAKTIK SEBAGAI KAWALAN BIOLOGI AGEN
TERHADAP PENYAKIT MATI ROSOT BETIK**

Oleh

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Betik (*Carica papaya*) adalah tanaman buah-buahan penting terhadap perkembangan ekonomi di Malaysia. Walau bagaimanapun, wabak penyakit mati rosot betik dalam industri betik telah menyebabkan pengeluaran betik merosot teruk. Matlamat kajian ini adalah untuk memencilkan serta mengenalpasti endofitik bacteria Asid Laktik daripada betik yang dapat merencatkan pertumbuhan *Erwinia mallotivora*, agen penyebab penyakit mati rosot betik. Kultur bacteria daripada ekstrak biji betik telah dicairkan secara bersiri dan disadurkan atas M17 agar dengan penambahan 0.5% glukosa. 18 daripada 70 koloni telah diuji positif yang dapat merencatkan pathogen ini menggunakan agar uji cakera resapan. Aktiviti antibakteria direkodkan dengan mengukur diameter (mm) zon perencatan yang jelas di sekeliling cakera. Pencilan bacteria A28 memperlihatkan zon perencatan jelas yang tertinggi iaitu 10 mm. Pencirian morfologi dan ujian biokimia terhadap pencilan yang dipencilkan menunjukkan bahawa mereka adalah Gram-positif, berbentuk kokus dan negatif katalase. Kaedah biologi molekul menggunakan penjujukan 16S rRNA masih berjalan. Pencilan mikrob yang terpilih di dalam kajian ini akan digunakan dalam mengendalikan penyakit Mati Rosot betik. Sepanjang pengetahuan kami, pendekatan biologi menggunakan bacteria Asid Laktik untuk mengawal penyakit ini masih belum diaplikasikan di Malaysia.

Kata kunci: Bacteria Asid Laktik, *Carica papaya*, Penyakit Mati Rosot Betik, *Erwinia mallotivora*

APPROVAL

This thesis was submitted to Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfilment of the requirement for the degree of Bachelor of Science (Hons.) Cell and Molecular Biology.

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DECLARATION

Declaration by graduate student

I hereby confirm that:

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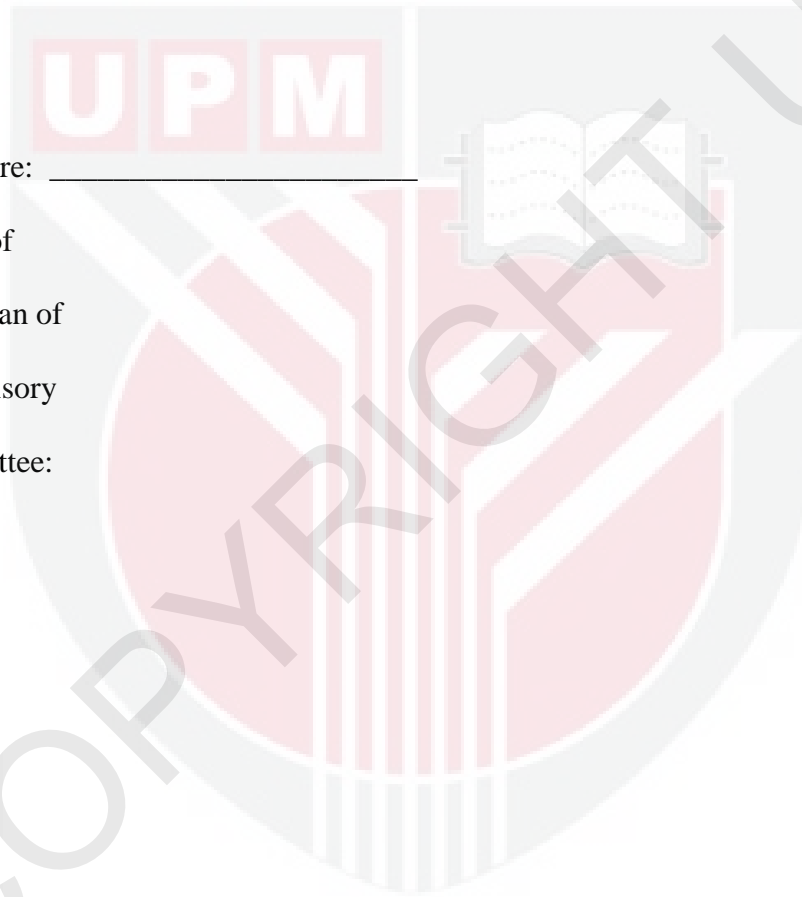
Declaration by Supervisor

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;

Signature: _____

Name of
Chairman of
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Committee:



ACKNOWLEDGEMENT

I am greatly indebted to a variety of people. I would like express my sincere attitude to my supervisor, Dr. Amalia Bt Mohd Hashim for her useful guidance, encouragement and continuous support throughout the duration of the research. Special thanks go to Mariam Dayana Bt. Mohd Taha and other senior post graduate students in Plant Molecular Biology Laboratory for guidance and helps towards this project.

Not forget, great appreciation goes to the rest of the seniors' laboratory member of the Cell and Molecular Laboratory that help me from time to time during the project. I also would like to thank to my colleagues for their moral support and advice in the laboratory. The whole time really brought us together to appreciate the true value of friendship and respect each other.

Last but not least, I would like to dedicate my gratitude and appreciation to my beloved mom (Hazizah bt Baharuddin), sisters (Nurul, Fauziah, Faridah and Fitrah) and little brother (Huzaidi) for their love, invaluable support and understanding during my research.

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LIST OF SYMBOLS AND ABBREVIATIONS

% (Pct)	Percentage
μl	microliter
$^{\circ}\text{C}$	Degree celcius
$\mu\text{g/ml}$	Microgram per millilitre
μM	Micro molar
x g	X gravity
BLAST	Basic Local Alignment Tool
bp	Base pair
BCP	bromocresol purple
c.f.u	Colony forming unit
DNA	Deoxyribonucleic acid
dH ₂ O	Distilled water
dNTPs	2'-deoxynucleotide 5' triphosphate
EDTA	Ethylene diamine tetacetic acid
EtOH	ethanol
g	Gram
g/L	Gram per litre
GM17	M17 media supplemented with 0.5 % (v/v) glucose
h	Hour
H ₂ O	Water
H ₂ O ₂	Hydrogen peroxide
L	Litre

LAB	Lactic acid bacteria
M	Molar
mA	Mili ampere
mg	Milligram
min	Minute
ml	mililitre
ml/L	Mililitre per litre
mM	Milimolar
mm	millimeter
NaOH	Sodium hydroxide
NaOCl	Sodium hypochlorite
NGS	Next-generation sequencing
O ₂	Oxygen
PCR	Polymerase chain reaction
PCI	Phenol-chloroform-isoamyl
rRNA	Ribosomal ribonucleic acid
RNA	ribonucleic acid
rpm	Rotation per minute
s	Second
SDS	Sodium dodecyl sulphate
V	Volt
v/v	Volume/volume
w/v	Weight/volume

CHAPTER 1

INTRODUCTION

1.1 Research background

Papaya (*Carica papaya*) belongs to the family of *Caricaceae* is an economically significant fruit crop grown in Malaysia. In 2004, Malaysia has become the second largest exporter among the papaya exporter countries after Mexico (Rahman *et al.*, 2007). Every part of papaya gives lots of benefits to the human. Besides, papaya fruit is a powerhouse of nutrient which is an excellent source of vitamins A, C and E (Aravind *et al.*, 2013).

Unfortunately, the emergence of Papaya Dieback disease has caused severe losses in papaya plantation in Malaysia. This disease was first found in Batu Pahat by Johor State Department of Agriculture around year 2003 before it has spread to the other states such as Perak, Malacca and Pahang (Amin *et al.*, 2011).

The major pathogen causing this disease is *Erwinia sp.* From the earlier study, the causative agent of this disease is *Erwinia papayae* (Maktar *et al.*, 2008), however recently *Erwinia mallotivora* has been detected as the new causative agent of Papaya Dieback disease in Peninsular Malaysia as reported by Amin *et al.*, (2011). The common symptoms caused by this plant pathogen are greasy, water-soaked lesions and spots on both leaves and fruit of the papaya plant.

In this study, we suggest that endophytic lactic acid bacteria in papaya might have a big potential to act as biological control agent against pathogenic bacteria. This research was inspired by the fact that lactic acid bacteria can control a number of plant pathogen (Ray *et al.*, 2012) and able to restrict the bacterial diseases (Sarr *et al.*, 2010). Moreover, lactic acid bacteria generally recognized as safe (GRAS) and has the ability to produce bacteriocin that can inhibit the growth of Gram negative bacteria (Alokomi *et al.*, 2000).

1.2 Problem statement

Currently, there are limited numbers of effective biological control approaches to treat Papaya Dieback disease caused by the pathogen, *Erwinia mallotivora*. Moreover, this disease has given major economic impacts on Malaysia as the biggest exporter for this unseasonal tropical fruit especially to the China and Singapore. Therefore this research was aimed to isolate endophytic LAB from healthy papaya with anti-bacterial properties against *E. mallotivora*, the causative bacterium of Papaya Dieback diseases.

1.3 Hypothesis

This study proposed that endophytic lactic acid bacteria from papaya have anti-microbial properties against the causative agent of Papaya Dieback disease, *E. mallotivora*.

1.4 Objectives of this study

- a) To isolate and screen endophytic lactic acid bacteria from healthy papaya's seed and sarcotesta.
- b) To determine endophytic lactic acid bacteria with antimicrobial activity against *E. mallotivora* by using agar overlay assay and agar disk diffusion assay.
- c) To characterize lactic acid bacteria using biochemical test: gram staining, catalase test and acidity test
- d) To identify the selected lactic acid bacteria with molecular method, 16s rRNA sequencing.