



UNIVERSITI PUTRA MALAYSIA

**PATHOGENICITY OF BANANA BLOOD DISEASE BACTERIUM HR2
MARDI AND CHARACTERIZATION OF ITS VIRULENT PROTEINS**

ROS AZRINAWATI HANA BAKAR

FP 2018 100



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By

ROS AZRINAWATI HANA BINTI BAKAR

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

July 2018

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DEDICATION

Special dedication to:

*This research work is dedicated to my beloved brother **Mohd Fazrullah Bakar** and sister in law, **Mrs Rahayu Ramdhan** also to my lovely sister **Rosazilawati Asma Bakar** for their excellence encouragement, compassion, guidance and constant prayers during the course of my study. In memory of my late father, **Bakar Bin Da** and my beloved late mother **Kalthum Bin Ismail**. The words could have been silent without both of you.*



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

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ROS AZRINAWATI HANA BINTI BAKAR

July 2018

Chairman : Associate Professor Khairulmazmi Ahmad, PhD
Faculty : Agriculture

In Malaysia, bacterial wilt has been identified as one of the main constraints for banana production. Blood Disease Bacterium (BDB) is a gram negative bacteria that caused Banana Blood Disease (BBD) which identified as one of the most important pathogen of banana (*Musa* sp.) which could infect almost all cultivated banana varieties. On the other hand, BDB is grouped into Phylotype IV which placed in a subgroup of the *R. solanacearum* species complex. BDB was reported to be pathogenic to a single host, banana. Hence, the first objectives of this study is to determine the host range and symptomatology study of BDB on selected hosts. Results from this study showed that BDB only pathogenic to banana not to other tested hosts such as tomato (*Solanum lycopersicum*) and heliconia (*Heliconia* sp.) and confirmed to be virulence and able to infect banana. The disease symptoms were wilting of leaves, discoloration of vascular tissues and eventually leads to plant death. The second objectives of this study is to identify and quantify effector protein(s) of BDB in selected media. From this study, findings indicated that 17 potential virulent proteins were obtained from virulence inducing media (M63). Prediction of virulence characteristics shows that the only five proteins were found to be effector proteins of BDB. The proteins were Signal peptidase, Lipoprotein, Uncharacterized protein, Membrane protein and Serine protease. In conclusion, BDB was confirmed to be a single host-pathogen to banana (*Musa* sp.) plant, and confirmed to be virulence for the proteomics study and based on proteomics analysis revealed five virulent proteins, namely Signal peptidase, Lipoprotein, Uncharacterized protein, Membrane protein and Serine protease associated with the virulence of BDB.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KEPATOGENAN PENYAKIT BAKTERIA DARAH PISANG HR2 MARDI DAN PENCIRIAN PROTEIN VIRULEN

Oleh

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Di Malaysia, penyakit layu bakteria telah dikenal pasti sebagai salah satu kekangan utama untuk pengeluaran pisang di Malaysia. Bakteria Penyakit Darah (BPD) ialah bakteria gram-negatif yang menyebabkan Penyakit Darah Pisang (PBP) dikenalpasti sebagai salah satu patogen yang penting bagi pisang (*Musa sp.*) yang menjangkiti hampir semua jenis pisang yang ditanam. Selain itu, BPD dikumpulkan dalam filotip IV dan diletakkan di dalam subkelompok kompleks spesies *R. solanacearum*. BDB dilaporkan sebagai patogen kepada perumah tunggal, pisang. Oleh itu, objektif pertama kajian ini adalah untuk menentukan jangkauan perumah dan kajian simptomologi BDB pada perumah terpilih. Keputusan dari kajian ini menunjukkan bahawa BDB hanya patogenik kepada pisang sahaja dan tidak kepada perumah lain yang diuji seperti tomato (*Solanum lycopersicum*) dan heliconia (*Heliconia sp.*) dan disahkan virulen dan berkebolehan menjangkiti pisang. Gejala penyakit ini ialah merosakkan daun, perubahan warna pada tisu vaskular dan akhirnya menyebabkan kematian kepada tumbuhan. Objektif kedua kajian ini ialah untuk mengenalpasti dan mengetahui kuantiti protein efektor BPD di dalam media terpilih. Hasil kajian menunjukkan 17 protein berpotensi virulen diperoleh daripada media perangsang virulen (M63). Ramalan terhadap ciri-ciri protein virulen menemukan hanya lima sahaja yang didapati sebagai protein efektor BPD. Protein virulen tersebut adalah protein isyarat peptidase, lipoprotein, protein yang belum dikenalpasti, protein membran dan protein serine. Kesimpulannya, BDB telah disahkan sebagai satu patogen tunggal untuk tumbuhan pisang (*Musa sp.*) dan berasaskan analisis proteomik mendedahkan lima protein virulensi iaitu protein isyarat peptidase, lipoprotein, protein yang belum dikenalpasti, protein membran dan protein serine dikaitkan dengan virulensi BDB.

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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 follow:

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
APS	Ammonium Per Sulphate
BBD	Banana Blood Disease
BDB	Blood Disease Bacterium
BLAST	Basic Local Allignment Search Tool
BSA	Bovine Serum Albumin
CPG	Casamino-acid Peptone Glucose
CS	Carbon Sources
CRD	Completely Randomized Design
cTP	Chloroplast Transit Peptide
DAI	Day After Inoculation
DH ₂ O	Distilled water
DOA	Department of Agriculture
EPP	Entry Point Project
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FASTA	FAST- All
FWHM	Full Width at Half Maximum
GNI	Gross National Income
GO	Gene Ontology
GPI-anchor	Glycosylphosphatidylinositol-anchor
GPI-SOM	Glycosylphosphatidylinositol Self Organizing Map
HCD	Higher Energy Collision Dissociation
HMM	Hidden Markov Model
HPLC	High Performance Liquid Chromatography
INIBAP	International Network for the Improvement of Banana and Plaintain
LCMS/MS	Liquid Chromatography Mass Spectrometry/Mass Spectrometry
MARDI	Malaysian Agriculture Research and Development Institute
MOA	Ministry of Agriculture

MS/MS	Tandem Mass Spectrometry
mTP	Mitochondrial Transit Peptide
MUDPIT	Multidimensional Protein Identification Technology
NB	Nutrient Broth
NCBI	National Centre of Biotechnology Information
NKEA	National Key Economic Area
NTP	Non Transmembrane Protein
pH	Potential of Hydrogen
SCX	Strong Cation Exchange
SDS PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel
SDW	Sterilized Distilled Water
SMART	Simple Modular Architecture Research Tool
SMSA	Semi Selective Medium South Africa
sP	Signal Peptide
SSPRED	Splice Site Prediction with Regulatory Element and Dependencies
SVM	Support Vector Machine
TargetP	Target Peptide
TCA	Trichloroacetic Acid
TEMED	Tetramethylethylenediamine
THMMM	Transmembrane Helices Hidden Markov Map
TMT	Tandem Mass Tags
Tris-HCL	Trisaminomethane Hydrochloric Acid
TTSS	Type Three Secretion System
TZC	Tetrazolium Chloride
USDA	United States Department of Agriculture
UV/VIS	Ultraviolet-visible Spectroscopy

LIST OF ANNOTATIONS

%	percent
kDa	Kilo Dalton
mL	Mililitre
w/v	Weight per Volume
v/v	Volume per Volume
rpm	Revolutions Per Minute
μ L	Microlitre
mM	Milimolar
mm	Milimetre
V	Volt
OD	Optical Density
nm	Nanometre
μ m	Micrometre
μ g	Nanogram
cm	Centimetre
mmt	Million Metric Tonnes
M	Molarity

CHAPTER 1

INTRODUCTION

Banana is one of the in-demand fruit in the world and possess among the top ten fruit commodities in Southeast Asia, Africa and Latin America (FAOSTAT, 2013). Bananas belong to *Musaceae* family under the genus *Musa* and categorised as perennial herbaceous monocots (Darvari et al., 2010). As reported by Sharrock and Frison,(1998) and INIBAP, (2002) shows that this fruit is the crucial staple foods due to the contribution in food security for over 100 million people in sub-Saharan Africa.

In Malaysia, banana (*Musa* spp.) is an economically important fruit and ranks one of the major planted fruit crops with 26,000 ha and 530,000 tonnes production per year (FAOSTAT, 2014). Banana was listed as the sixth high-value non-seasonal tropical fruits crop alongside papaya, pineapple, rock melon, star fruit and jackfruit under the Entry Point Project (EPP) of the National Key Economic Area (NKEA) for premium fruit production (Ministry of Agriculture, 2015). As stated by FAOSTAT, (2005) bananas placed as fourth vital fruit crops as banana provides food to millions of people in South East Asia. Malaysia is one of the origin country of banana which has a great variety of banana species, around 50 types including the commercial varieties such as Berangan, Rastali, Nangka and Abu Nipah which belong to three genome types in *Musa* (AAA, AAB, and ABB) (Darvavi et al., 2010). Notably, cash crops industry including fruits and vegetables contributed to about 27% of Malaysia Gross National Income (GNI). Banana is also considered as a super food rich in minerals, energy and nutrients. Reported by USDA, (2004) approximately 100 g of raw banana flesh contain 122 Kcal of energy, which provides 1.30 g of proteins, 0.37 g of fat, 0.6 mg of iron, 0.14 mg of zinc and 457 µg of β-carotene. As with any other crops, banana is infected with many diseases which eventually leads to the reduction of yield and quality of the fruit produces worldwide.

In Malaysia, bacterial wilt disease is caused by several phytopathogens which have a broad range of hosts. One of the most important diseases in bananas is banana blood disease (BBD). It is the destructive disease of bananas in Malaysia. This disease is caused by bacteria pathogen that causes quality and yield decline in banana production. Report from FAOSTAT, (2014), showed that in 2007 the yield of banana production decrease to about 100,000 tonnes per year. Recent survey carried out by the Department of Agriculture (DOA) in state of Johor, stipulated that 61% of the banana plants in the surveyed areas (3200 ha) were affected by the BBD. The causative pathogen is Blood Disease Bacterium (BDB).

According to Fegan and Prior, (2005) BDB is belongs to the phylotype IV of the *Ralstonia solanacearum* species complex. The disease symptoms includes wilting of leaves, discoloration of vascular tissues, fruits and eventually leads to plant death.

To date there is no resistant commercial banana plant to the pathogen was reported. Of now, the details of epidemiology, pathogenesis and host-pathogen interaction of the pathogen are not very well understood.

This study is emphasising on the host range of the BDB as this pathogen was reported to be a single host pathogen to only infect banana species but do not infect other species of plants (Remenant et al., 2011). The pathogenicity assay is the only non-molecular test that help to differentiate between Moko disease and BBD. However, both diseases produce almost identical disease symptoms, which were yellowing and wilting of leaves and eventually leads to plant death.

To further understand the biology of the pathogen, research was carried out to discover the effector proteins associated with virulency of BDB. Effector proteins is a secreted proteins that are responsible in pathogenesis and virulency of a pathogen (Green & Mecsas, 2016). In this study, computational prediction using bioinformatics tools were also carried out to discover and explore bioinformatics of BDB via examining *in silico* data of the proteins function. This data is very important fundamental information for the development of disease resistant variety of banana.

The aim of this study were to determine the hosts range and symptomatology of BDB and its virulent protein(s) that associated with the pathogenicity of BDB, therefore the specific objectives of this work were:

- i. To determine the hosts range and symptomatology of BBD.
- ii. To identify and quantify potential effector protein(s) of BDB in selected media.

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