



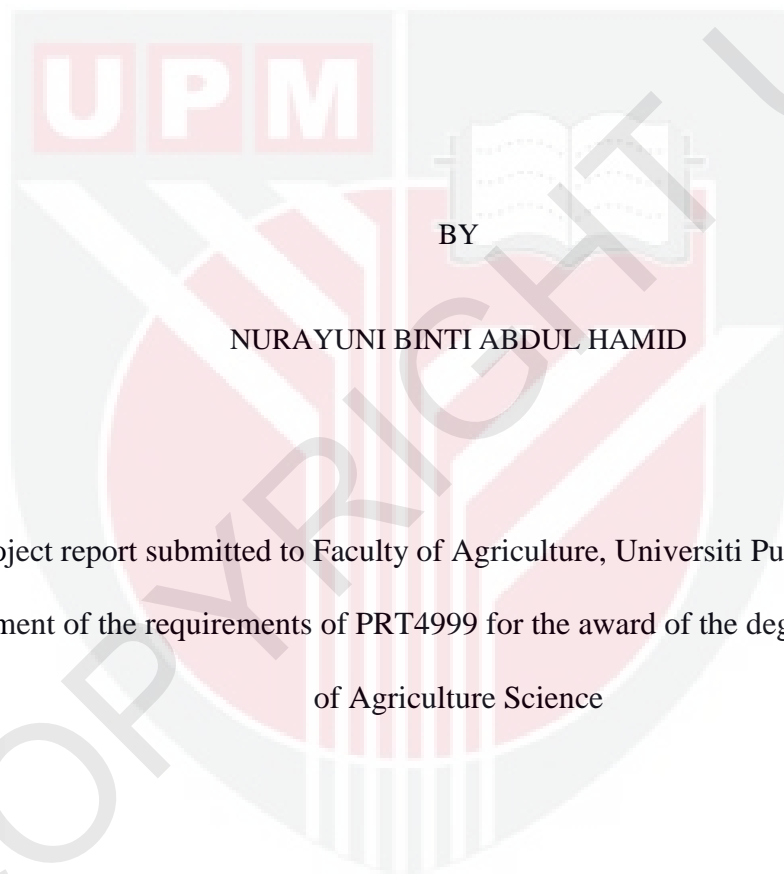
UNIVERSITI PUTRA MALAYSIA

**ASSESSMENT OF ENDOPHYTIC BACTERIA FOR SUPPRESSION OF
RALSTONIA SOLANACEARUM GROWTH *INVITRO***

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ASSESSMENT OF ENDOPHYTIC BACTERIA FOR SUPPRESSION OF
RALSTONIA SOLANACEARUM GROWTH *INVITRO*.



BY

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fulfilment of the requirements of PRT4999 for the award of the degree of Bachelor
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CERTIFICATION

This research paper entitled '**Assessment of Endophytic Bacteria For Suppression of *Ralstonia solanacearum* Growth Invitro** ' is prepared by Nurayuni binti Abdul Hamid and submitted to the Faculty of Agriculture as partial fulfilment of the requirement for the PRT4999 (Research Project) for the award of the degree in Bachelor Science of Agriculture.

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TABLE OF CONTENTS

ACKNOWLEDGEMENT.....	i
TABLE OF CONTENTS.....	ii
LIST OF TABLES/FIGURES/APPENDICES.....	iv
ABSTRACT.....	vi
ABSTRAK.....	viii
CHAPTER 1: INTRODUCTION.....	1
CHAPTER 2: LITERATURE REVIEW	
2.1: Bacteria wilt diseases.....	4
2.2: Symptoms/indication of bacterial wilt.....	7
2.3: Mode transmission and spread of bacterial wilt.....	9
2.4: The control of bacteria wilt.....	12
2.5: Bacterial antagonist against <i>R. solanacearum</i>	15

CHAPTER 3: MATERIALS AND METHODS

3.1: Collection of samples of wilt affected plants..... 18

3.2: Isolation of the pathogen *R. solanacearum*..... 21

3.3. Detection and characterization of the pathogen..... 22

R solanacearum.

3.4. Isolation of antagonistic bacterial strains..... 22

3.5. In-vitro evaluation of bacterial isolates against *R. solanacearum*..... 23

CHAPTER 4: RESULTS & DISCUSSION

4.1. Isolation of wilt-causing bacteria, *R. solanacearum*..... 24

4.2. Isolation of the Endopytic bacteria..... 27

4.3. Effect of different concentration on antagonistic..... 32

bacteria on suppression of *R.solanacearum*

CHAPTER 5: CONCLUSION.....34

REFERENCES..... 35

APPENDICES..... 39

LIST OF TABLES **Page**

Table 1: Cultural colony characteristics of the bacteria..... 29
on Nutrient Agar (NA) media

Table 2: Antagonistic activity of five antagonistic bacterial..... 33
isolates against *R.solanacearum*.

Table 3: Antimicrobial susceptibility test interpretive category..... 33

LIST OF APPENDICES

Appendix 1: Media for isolation and cultivation of..... 35
Ralstonia solanacearum

Appendix 2: Tripheny tetrazolium chloride (TTC or TZC) medium..... 36

Appendix 3: Concentration of antagonistic bacteria..... 37

LIST OF FIGURES	Page
Figure 1: Symptoms of Moko disease on <i>Musa paradisiaca</i> 8 cv. Nipah examined in this study.	
Figure 2: The leaves are yellowing and wilt, which..... 18 then become necrotic and collapse.	
Figure 3: The vascular tissue will turns from brown..... 19 to black in colour	
Figure 3: The fruit skin remain green but the flesh 19 change to brownish discoloration	
Figure 4: The infected banana stem discharging the..... 20 bacterial exudates called bacterial ooze.	
Figure 5: Morphological characteristic of the avirulent colony..... 25 of <i>R. solanacearum</i> on Kelman's TZC media.	
Figure 6: Morphological characteristic of the colony of suspected a..... 26 virulent wild-type <i>R. solanacearum</i> on Kelman's TZC media.	
Figure 7: The isolate of antagonistic bacteria on NA (Ps8)..... 28	
Figure 8: The antibacterial activity showing inhibition zones of..... 31 antagonistic bacteria isolates against <i>R. solanacearum</i> isolates from healthy plants on MHA.	

ABSTRACT

An abstract of the research paper presented to the Faculty of Agriculture in partial requirements on the course PRT4999-Research Project.

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By

Nurayuni Binti Abdul Hamid

2015/2016

Dr. Khairulmazmi Bin Ahmad

Banana (*Musa spp.*) is an important fruit crop Malaysia and grown for fresh and process fruits. Bacterial wilt disease or Moko caused by *Ralstonia solanacearum* is one of a serious diseases in bananas and plantains cultivation worldwide. Although it is a major threat to the banana cultivation, there is no effective control measure has been developed against it yet. In view of this research was conducted i) to isolate *R. solanacearum*, the causal pathogen of the bacterial wilt disease and ii) to screen endophytic bacteria for the selection of effective biocontrol agents. Diseased samples were taken from the areas that has been reported was infected by the disease in Johor.

The bacterial pathogen was isolated using Kelman's Selective Media, Tetrazolium chloride (TZC) Medium and subsequently maintained onto Casamino acid-Peptide-Glucose (CPG) medium. Antagonistic bacteria were screened *in vitro* by using agar diffusion well test. There are ninety eight endophytic bacteria were screened for their antibacterial activity against *R. solanacearum* by measuring the inhibition zone. At the end of study, only one isolate was found suppress the growth of *R. solanacearum in vitro*. However, the isolate is not susceptible to be chosen as one of the effective biological control against the pathogen, *R. solanacearum*.

Keywords: Biological control; Antagonist; Endophytic bacteria; *Ralstonia solanacearum*; Bacterial wilt

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Pertanian untuk memenuhi sebahagian keperluan kursus PRT4999- Projek Penyelidikan.

PENILAIAN ENDOFIT BAKTERIA UNTUK MENYEKAT PERTUMBUHAN *RALSTONIA SOLANACEARUM* SECARA INVITRO.

Oleh

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Dr. Khairulmazmi Bin Ahmad

Pisang (*Musa spp.*) adalah tanaman buah-buahan yang penting untuk Malaysia dan ditanam untuk buah-buahan segar dan proses. Penyakit Moko ataupun layu bakteria adalah disebabkan oleh *Ralstonia solanacearum* adalah salah satu penyakit yang serius pada pisang dan penanaman plantain di seluruh dunia. Walaupun ia adalah ancaman utama kepada penanaman pisang, namun masih belum ada langkah kawalan yang efektif telah dibangunkan terhadapnya lagi.

Sehubungan itu, kajian ini dijalankan bertujuan untuk i) untuk mengasingkan dan mengenalpasti *R. solanacearum* iaitu patogen penyebab bagi layu bakteria atau penyakit Moko dan ii) untuk skrinkan bakteria endofitik untuk pemilihan agen kawalan biologi yang berkesan. Sampel berpenyakit akan diambil dari kawasan-kawasan yang telah dilaporkan telah dijangkiti penyakit ini di Johor. Patogen bakteria akan diasingkan dengan menggunakan agar terpilih Kelman iaitu media klorida tetrazolium (TZC) dan kemudiannya akan dikekalkan ke media Casamino asid-pepton - Glukosa (CPG). Sementara itu, abakteria antogonis diskrikan secara *invitro* dengan menggunakan kaedah resapan telaga agar dengan mengukur kawasan penahanan. Sebanyak sembilan puluh lapan abakteria antogonis telah diskrikan untuk aktiviti antibakteria terhadap *Ralstonia solanacearum*, agen penyebab penyakit layu bakteria. Di penghujung kajian, didapati satu isolate abakteria antogonis telah berkesan untuk menghalang petumbuhan *R. solanacearum in-vitro*. Namun, isolate tersebut tidak mempengaruhi untuk dipilih sebagai agen kawalan biologi yang efektif terhadap patogen, *R. solanacearum*.

Kata kunci: Kawalan Biologi, Abakteria Antogonis, *Ralstonia solanacearum*, Layu Bakteria.

CHAPTER 1

INTRODUCTION

Banana (*Musa spp.*) is one of the important fruit crops in Malaysia and secondly most widely cultivated fruit crop that covering about 26,000 ha with a total production of 530,000 metric tonnes. There are about 15% of the production were exported to Singapore, Brunei, Hong Kong and Middle East valued at more than US\$10 million. In 2009, the major producing states are Johor, Sabah and Sarawak for a total of 27,543 hectares. These areas are expected to expand in the future due to increasing local and foreign demand and newly implemented Economic Transformation Programme (ETP) by the government (MARDI, 2013).

Although bananas and plantains can adapt efficiently to produce high yields under a wide range of climatic extremes, they are susceptible to a range of serious and debilitating diseases (Robinson *et al.*, 2010). Thus, disease can be a one of the important limiting factors that can significantly lower down the production of banana in Malaysia and had been continued to be a devastating disease in many countries (IPDN, 2014). The emerging of the new bacterial wilt disease, has further dampened the banana industry. Bacterial wilt of banana was ranked as number one as one of the very important diseases that influence the banana production.

This disease is caused by *Ralstonia solanacearum* that will cause a severe wilting of the plants (DOA, Australia, 2005). This disease was first observed to be attacking banana on 16 March 2007 in Pontian, Johor as it follows a serious flood that hit the state in the same year (MARDI, 2013).

R. solanacearum is an important soil borne bacterial plant pathogen which is a widely distributed plant disease in tropical, subtropical and warm temperate regions of the world (Hayward, 1991).

The bacterium, which is often endemic in the soil, penetrates the plant through the root system and eventually causes irreversible wilting and death of the plant (IPDN, 2014). This bacteria can survive in soil for almost two years or more (MARDI, 2013). It can also be spread by water, soil, farm implements and machinery and it will spread rapidly by transmission by insects.

In the Amazon Basin in Peru, about half the banana plantations are affected and the rapid spread of the pathogen threatens to destroy plantations throughout the Peruvian jungle (French & Sequeira, 1968; Burjein, 2015). The yield loss estimated can be up to 100% as all of the plant parts will be infected by *R. solanacearum* as well as the fruit. The fruit infected by *R. solanacearum* will show the brown discoloration thus, the whole plant must be destroyed (MARDI, 2013).

Conventional management strategies like crop rotation, adjusting the date of planting, cultural methods and soil treatment are not effective (Chellemi et al. 1997; R. Ramesh et al., 2008). Resistant cultivars are either location-specific or generally not preferred by growers due to low consumer preference. Thus, control of *R. solanacearum* seems to be difficult due to lack of universal control treatments (Hayward 1991; R. Ramesh et al., 2008), and to date no effective control method exists for bacterial wilt disease (R. Ramesh et al., 2008).

Recent studies have indicated that biological control of bacterial wilt disease could be achieved using antagonistic bacteria (R. Ramesh *et al.*, 2008). Hallmann *et al.* (1997) defined an endophyte as any microorganism that resides inside the plant without regard to the specific tissue colonized and these bacterial endophytes can be isolated from surface-disinfected plant tissue or extracted from internal plant tissue (R. Ramesh *et al.*, 2008).

Although endophytes were already described in the past century, they have received considerable attention only in the past decade when their capacity to protect their hosts against biotic factors was recognized (R. Ramesh *et al.*, 2008).

Endophytic bacteria is a particular bacterial strains in certain natural environments prevent infectious diseases of plant. Endophytic bacteria live in plant tissues without doing substantive harm or gaining benefit other than residency (Kobayashi & Palumbo, 2000; R. Ramesh *et al.*, 2008).

During colonization, these bacteria produce antibiotics, elicit induced systemic resistance in the host plant or interfere specifically with pathogenicity factors. Before engaging in these activities, biocontrol bacteria go through several regulatory processes at the transcriptional and post-transcriptional levels (Haas *et al.*, 2005). Some of the bacteria such as *Pseudomonas fluorescens*, *P. alcaligenes*, *P. putida* been reported as endophytes and were inhibitory to plant pathogens (R. Ramesh *et al.*, 2008). This thesis will be discussing on the isolate of *R. solanacearum*, the causal pathogen of the bacterial wilt disease and to screen endophytic bacteria for antagonistic activity and growth, the biocontrol activity against the bacterial wilt pathogen *invitro*.

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