UNIVERSITI TEKNOLOGI MARA

THE POTENTIAL OF ENDOPYTIC BACTERIA AS PLANT GROWTH PROMOTER AND BIOCONTROL AGENT FOR BACTERIAL LEAF BLIGHT

RASHIDAH BINTI ABD HALIM

Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science
(Applied Biology)

Faculty of Applied Sciences

April 2022

ABSTRACT

Excessive use of synthetic agents to control bacterial leaf blight disease in rice hinders with environmental and health issues. Endophytic bacteria, which can be recovered from inside plant tissues such as roots, stems, and leaves, might overcome this limitation due to their unique ecological niche inside plant roots, where they are sheltered from external environmental disturbances. Some of these bacterial endophytes have beneficial effects on their host plants and stimulate plant growth or reduce disease symptoms, apparently through mechanisms that are similar to those proposed for plant-growth promoting rhizobacteria (PGPR). The objective of this study was to assess a collection of endophytic bacteria from the roots and leaves of rice plants and explore the potential used to control bacterial leaf blight and plant growth promoting (PGP) traits. Two hundred and twelve isolates were obtained from the roots and leaves of rice samples collected from different rice cultivation fields across Peninsular Malaysia. Morphological and biochemical analysis grouped these isolates into 6 distinct groups. Further, 16S rRNA gene sequencing analysis identified these isolates as *Bacillus cereus* strain DQ884352.1, *Bacillus subtilis* strain KF957733, *Geobacillus thermoparaffinivorans* strain KP218042.1, *Pseudomonas fluorescens* strain MH620728.1, *Enterobacter* sp. strain CP028975, and *Gamma proteobacterium* strain CP032508.1. All of these isolates exhibited various abilities to produce growth regulators that contributed to solubilizing phosphate, produced indole-3-acetic acid (IAA), synthesised siderophores, were able to fix nitrogen, and showed antagonistic activities against *Xanthomonas oryzae* pv. *oryzae*, pathogenic bacteria under *in vitro* conditions. All the six endophyte isolates were further evaluated for their ability to enhance plant growth and disease suppression efficiency under greenhouse conditions. Results revealed that *Geobacillus thermoparaffinivorans* strain KP218042.1 significantly ($p < 0.05$) enhanced the root length (29.73 ± 1.55 cm) and showed a significantly ($p < 0.05$) maximum grain yield (34.23 ± 7.79). Disease treatment experiments showed that the suppression efficiency of *Geobacillus thermoparaffinivorans* strain KP218042.1 treatments was 83.49 %, which was significantly higher ($p < 0.05$) than that of the bactericide treatment (24 %). These results suggest that *Geobacillus thermoparaffinivorans* strain KP218042.1 may be useful as biofertilizers and/or biocontrol agents in sustainable agricultural practices.

ACKNOWLEDGEMENT

Firstly, Alhamdulillah, in the name Allah S.W.T. the most merciful, the most gracious, the most powerful of all, with His blessing for giving me the opportunity to embark on my Master's Degree program and for completing this long and challenging journey successfully. My gratitude and thanks goes to my main supervisor, Dr Nor'Aishah bt Hasan for her encouragement as well as many hours of attention and guidance she has devoted to this research. I'm so proud to have her as my supervisor for helping me a lot and giving full commitment on her responsibility.

I also would like to acknowledge and extend my heartfelt gratitude to Madam Kogeethavani as my co-supervisors who never tired in teaching me everything and giving me chances to finish this project successfully. Thank you very much. May Allah grant both of them something special for their kindness.

My appreciation goes to the Assistant Science officer in MARDI, Fatin Nurliyana binti Ahmad, Muhamad Iswan bin Muhamad Fazil and Nur Syahirah binti Ahmad Sobri who provided the facilities and assistance throughout the research. Without them, I may not be able to make it this alone by myself, thank you. Special thanks to my colleagues and friends for the continuous support throughout this project. Big thanks also I wish for buddy, Nor Afeeqah bt Zannudin, who is really helping me and giving me knowledge about this project.

Finally, this thesis is dedicated to the loving memory of my very dear father and mother, Abd Halim bin Md Lazim and Jamilah binti Hj Ramli and also my siblings for their love, vision, determination, understanding, endless support and prays along my study to educate me. This piece of victory is dedicated to them. Alhamdulillah.

TABLE OF CONTENTS

Page

CONFIRMATION BY PANEL OF EXAMINERS **ii**

AUTHOR'S DECLARATION **iii**

ABSTRACT **iv**

ACKNOWLEDGEMENT **v**

TABLE OF CONTENTS **vi**

LIST OF TABLES **ix**

LIST OF FIGURES **x**

LIST OF PLATES **xi**

LIST OF ABBREVIATIONS **xii**

CHAPTER ONE INTRODUCTION **1**
1.1 Research background 1
1.2 Problem statement 3
1.3 Research objectives 4
1.4 Significance of study 4
1.5 Limitation of study 5

CHAPTER TWO LITERATURE REVIEW **6**
2.1 General introduction 6
2.2 The bacterial leaf blight pathogen *Xanthomonas oryzae pv. oryzae* (*Xoo*) 8
2.3 Management practices of rice bacterial leaf blight 10
2.4 Endophytic bacteria 12
2.4.1 Diversity of endophytic bacteria 13
2.4.2 Endophytic bacteria in rice plant 16
2.5 Colonisation of endophytic bacteria 20
2.6 Application of Endophytes 22
2.6.1 Direct growth promotion mechanism 24
2.6.1.1 Phytohormone IAA production 24
2.6.1.2 Phosphate solubilization 24
2.6.1.3 Siderophores synthesis 25
2.6.1.4 Biological nitrogen fixation 26
2.6.2 Indirect growth promotion mechanism 27
2.6.2.1 Biological control of plant pathogens 28
2.7 Bacterial identification and classification 29
2.7.1 Molecular analysis of endophytic diversity 30

CHAPTER THREE METHODOLOGY **32**
3.1 Introduction 32

CHAPTER ONE
INTRODUCTION

1.1 Research background

Rice (*Oryza sativa* L.) is a key source of carbohydrate-based nourishment and one of the world's most important staple foods, various vitamins and minerals (Rathna *et al.*, 2019). It is farmed on approximately a significant acreage of arable land in Asia, and China remains one of the world's top rice-producing countries (Zahra *et al.*, 2018). According to projections, the world's population will increase by 2.3 billion (or 34%) by 2050. Moreover, the average food consumption is also expected to rise from 2000 to 3070 kcal per person per day. To meet these demands, rice production has to be increased by 60% over the next 40 years (FAO, 2012). It will be the key approach for reducing the global food insecurity over the coming decades (Firdaus *et al.*, 2020).

Rice is a major source of employment and income for rural farmers in Malaysia, with the average Malaysian resident consuming 82.3 kg of rice each year (Raweekul, 2016). Malaysia's paddy supply is mostly based on 10 primary rice granaries, with an average national output of roughly 4.0 MT/Ha (Omar *et al.*, 2019). Unfortunately, similar to other places of the world, in Malaysia, rice production is highly susceptible to environmental and threatened by phytopathogens (Muehe, 2019). One of the most common rice diseases is bacterial leaf blight (BLB), caused by the bacteria pathogen *Xanthomonas oryzae* pv *oryzae* (*Xoo*), which levelling off the rice yield production and plant growth due to the extensive range of biochemical, molecular and physiological modification of the rice plants (Khan *et al.*, 2019). The capability of the pathogen to grow under conducive environment had resulted more than 50% of yield losses worldwide including Malaysia (Chukwu *et al.*, 2019). It is difficult to find remedies to fight the pathogen, reduce the loss of rice yield and thereby mitigate the danger to global food security. Commercial fertilizers and pesticides have been used as major approaches for control the bacteria pathogen (Vu and Oh, 2019). However, these strategies are not effective, non-environmentally friendly neither practical. Biofertilizers and biopesticides may become the preferred substitutions for some conventional synthetic products. Unfortunately, bacterial products can show some