



UNIVERSITI PUTRA MALAYSIA

**ANTIMICROBIAL POTENTIAL OF ENDOPHYTIC BACTERIA AGAINST
BACTERIAL LEAF BLIGHT, *Xanthomonas oryzae* pv. *oryzae***

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FACULTY OF AGRICULTURE
UNIVERSITI PUTRA MALAYSIA

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BY

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CERTIFICATION

This project report entitled “Antimicrobial potential of endophytic bacteria against bacterial leaf blight (*Xanthomonas oryzae pv. oryzae*)” was prepared by Aqmal Asyraf bin Salleh and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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TABLE OF CONTENTS

CONTENTS	PAGE
CERTIFICATION	i
ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF APPENDICES	vii
LIST OF ABBREVIATIONS	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER	
1 INTRODUCTION	
1.1 Introduction	1
1.2 Objectives of Study	3
1.3 Significant of Study	3
2 LITERATURE REVIEW	
2.1 Host: Rice	4
2.2 Rice Production in Malaysia	5
2.3 Rice Diseases in Malaysia	6
2.4 Bacterial Leaf Blight (BLB)	8
2.4.1 Bacterial leaf blight (BLB) in Malaysia	9

2.5	Management of bacterial leaf blight (BLB)	
2.5.1	Chemical control	9
2.5.2	Cultural control	10
2.5.3	Endophytic bacteria as biological control method	11
3	MATERIALS AND METHODS	
3.1	Sample collection	12
3.2	Isolation of Xoo	12
3.3	Isolation of endophytic bacteria	13
3.3.1	Isolation from rhizosphere	13
3.3.2	Isolation from plant parts	14
3.4	Dual culture test	
3.4.1	Preparation of bacterial extract	15
3.4.2	Preparation of Xoo broth culture	15
3.4.3	Assay of antimicrobial activity	16
3.5	Statistical analysis	16
4	RESULTS	
4.1	Isolation of pathogenic bacteria	17
4.2	Isolation of endophytic bacteria	18
4.3	Assay of antimicrobial activity	20
5	DISCUSSION	23
6	CONCLUSION	25
	REFERENCES	26
	APPENDICES	29

LIST OF TABLES

TABLE		PAGE
Table 1	Isolated bacteria colonies and distinguished morphological characteristics on peptone sucrose agar (PSA)	18
Table 2	Colonies of endophytic bacterial strains	18
Table 3	Endophytic isolates colony distinguished characteristics on nutrient agar (NA) media	19
Table 4	Inhibition zone (mm) of bacteria D1 and bacteria B3 against pathogen, <i>Xanthomonas oryzae pv. oryzae</i>	20
Table 5	Inhibition zone (mm) of control variables against pathogen, <i>Xanthomonas oryzae pv. oryzae</i>	21

LIST OF FIGURES

FIGURES	PAGE
Figure 1 Xanthomonads translucens growing on peptone sucrose agar (PSA) showing yellow pigment	17
Figure 2 Antimicrobial assay Bacteria D1 against <i>Xanthomonas oryzae pv. oryzae</i>	21
Figure 3 Antimicrobial assay Bacteria B3 against <i>Xanthomonas oryzae pv. oryzae</i>	21
Figure 4 Control variable of antimicrobial assay against <i>Xanthomonas oryzae pv. Oryzae.</i>	22
Figure 5 Regression of different concentration of bacterial extract on inhibition size against <i>Xanthomonas oryzae pv. oryzae.</i>	24

LIST OF APPENDICES

APPENDIX		PAGE
Appendix 1	ANOVA TABLE - The effectiveness of the ethyl acetate bacteria B3 and D1 extract against <i>Xanthomonas oryzae pv. oryzae</i> .	29
Appendix 2	ANOVA TABLE - The effectiveness of different concentrations of the ethyl acetate bacteria B3 and D1 extract against <i>Xanthomonas oryzae pv. oryzae</i> .	30
Appendix 3	ANOVA TABLE - The comparisan of each concentrations of the ethyl acetate bacteria B3 and D1 extract against <i>Xanthomonas oryzae pv. Oryzae</i> using Tukey test. Analysis	32

LIST OF ABBREVIATIONS

°C	degree celcius
%	percentage
ANOVA	analysis of variance
BLB	bacterial leaf blight
BSB	bacterial sheath blight
CRD	complete randomized design
g	gram
ha	hectare
LBD	leaf blast disease
MHA	Mueller hinton agar
ml	mililitres
NA	Nutrient agar
NB	Nutrient broth
PSA	Peptone sucrose agar
rpm	revolutions per minute
SAS	Statistical analysis system
w/v	weight per volume
Xoo	<i>Xanthomonas oryzae pv. oryzae</i>

ABSTRACT

Rice is perhaps the most widely cultivated food crop world over, whose production is constrained by diseases of fungal, bacterial and viral origin. The bacterium *Xanthomonas oryzae* pv. *oryzae* (Xoo) or previously known as *Xanthomonas campestris* pv. *oryzae* for example, is the causal pathogen of the bacterial leaf blight disease (BLB) of rice in Asia (Swings et al. 1990). Endophytic bacteria may have several potential applications in various sectors of biotechnology including agriculture. Bacterial endophytes need to be explored for their potential applications in agricultural biotechnology. Hence, this study was undertaken to explore the antagonistic antimicrobial potential of endophytes isolated from rice against the pathogenic bacteria, *Xanthomonas oryzae* pv. *oryzae*. In this study, plant samples were collected from rice field of Integrate Agriculture Development Area (IADA) Barat Laut in Sawah Sempadan, Kuala Selangor, Malaysia. Pathogenic bacteria, Xoo were isolated using Peptone Sucrose Agar (PSA) media. Endophytic bacteria were isolated using Nutrient Agar (NA) media via serial dilution method from different parts of rice such as root, sheath, leaves, and root rhizosphere. Potential endophytic bacteria screened by dual culture test were conducted using agar well diffusion method (Azoro et al., 2002) in Mueller Hinton Agar (MHA) media. Endophytic bacterium B3 and D1 showed positive inhibition against Xoo. The two bacteria showed activity against tested bacterial pathogen but in a weak state. The isolates are not susceptible to be chosen as one of the effective biological control against Xoo.

ABSTRAK

Padi adalah antara tanaman makanan dunia yang paling banyak ditanam diseluruh dunia dimana pengeluarannya dikekang oleh penyakit kulat, bakteria, dan virus. Bakteria *Xanthomonas oryzae pv. oryzae* (Xoo) atau sebelum ini dikenali sebagai *Xanthomonas campestris pv. oryzae* sebagai contoh, adalah patogen yang menjadi penyebab kepada penyakit hawar daun (BLB) pada tanaman di Asia (Swings et al. 1990). Bakteria endofitik mungkin mempunyai beberapa aplikasi yang berpotensi dalam pelbagai sektor bioteknologi termasuk pertanian. Endofitik bakteria perlu diterokai untuk aplikasi potensi mereka dalam bidang bioteknologi pertanian. Oleh itu, kajian ini dijalankan untuk meneroka potensi anti-mikrob yang antagonistik daripada bakteria endofitik yang diasingkan daripada tanaman padi terhadap patogen, *Xanthomonas oryzae pv. oryzae*. Dalam kajian ini, sampel tumbuhan akan diambil dari kawasan sawah padi Kawasan Pembangunan Pertanian Intergrasi (IADA) Barat Laut, Sawah Sempadan, Kuala Selangor, Malaysia. Bakteria patogenik, Xoo diasingkan menggunakan agar pepton sukros (PSA) media. Bakteria endofitik diasingkan menggunakan agar nutrien (NA) media melalui kaedah pencairan bersiri dari pelbagai bahagian tanaman padi seperti akar, sarung, daun, dan akar rhizosfera. Bakteria endofitik yang berpotensi akan disaringkan menggunakan kaedah agar well diffusion (Azoro et al., 2002) dalam media Mueller Hinton Agar (MHA). Bakteria endofitik B3 dan D1 menunjukkan perencatan positif terhadap Xoo. Kedua-dua bakteria mampu merencat backteria patogenik tetapi dalam keadaan lemah. Isolate tersebut tidak sesuai dipilih sebagai salah satu daripada kawalan biologi yang berkesan terhadap Xoo.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) or early known as *Xanthomonas campestris* pv. *Oryzae* is a very devastating disease of rice throughout the world. BLB is the most serious disease in South Asia (Ou, 1985) which was first reported in Fukuoka Prefecture, Japan, during 1884. Subsequently, its incidence has been reported from different parts of Asia, northern Australia, Africa and USA. The disease was reported in South East Asia in the early 1960s, where it is currently widespread, and it still affects the rice crop in its severe form (Goto, 1992). It causes yellowing of the leaves, followed by death of infected leaf tissues, which usually starts from the tip of the leaves which causes the reduction in the leaf area size, resulting severe yield reduction of up to 50% (Saad 1995).

The severity and significance of damages caused by infection have necessitated the development of strategies to control and manage the disease, so as to reduce crop loss and to avert an epidemic. The common approach for control rice disease is using chemical fertilizer. Chemical fertilizer such as pesticides, herbicides, and insecticides has a negative effect such as decrease the soil microflora, induce resistance of pathogens, and the residue may affect to humans and animal's health. For example, the use of chemical substance like Bordeaux mixture, Cu and mercurial compounds.

Extensive use of these chemical causes harmful impacts on human and environment. The alternative to substitute chemical fertilizer is use of endophytic bacteria.

Endophytic bacteria are defined as those bacteria that can be isolated from surface-disinfected plant tissues or extracted from within the plants and that are not observed to harm the host plants. Endophytic bacteria colonize healthy plant tissue without causing symptoms or damages to the host (Hallman et al. 1997). They can be isolated from internal plant tissue after thorough surface-disinfection of the plant tissue, either from herbaceous or woody plants (Taechowisan et al. 2003b; Cao et al. 2004; Inderiati & Franco 2008). Endophytic microorganisms provide advantages to the host plant by enhancing the physiological activity of the plant or through other modes of action and thus may serve as a source of agroactive compounds, biocontrol agents, or plant growth promoters (Shimizu et al. 2009; Dombou et al. 2002). Species of bacteria like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance the plant growth (Kloepper et al., 1989, Okon and Labandera-Gonzalez, 1994, Glick, 1995 and Gururani et al., 2012).

1.1 Objectives of study

The objectives of this study is to assess the *in-vitro* antimicrobial activity of endophytic bacteria isolated from rice against *Xanthomonas oryzae pv. oryzae*.

1.2 Significant of study

There are no effective controls of the disease bacterial leaf blight (BLB) which is caused by *Xanthomonas oryzae pv. oryzae*. The control method which usually used is chemical fungicide for example the use of to control BLB is by spraying copper oxychloride and streptomycin solution which inhibits the growth of the bacteria. In this case, an effective biological control is important in order to control the BLB disease without relying too much on chemicals. At the same time, the environment and the biodiversity can be conserved which currently is degrading because of the excessive usage of chemical pesticides which is harmful to people and other living organisms.

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