UNIVERSITI PUTRA MALAYSIA

ANTIBACTERIAL ASSAY AND MOLECULAR IDENTIFICATION OF SELECTED ANTAGONISTIC ACTINOMYCETE STRAIN AGAINST BACTERIAL LEAF BLIGHT DISEASE PATHOGEN, Xanthomonas oryzae pv. oryzae

NURUL FATIAH BINTI SHABUDIN

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NURUL FATIAH BINTI SHABUDIN

FACULTY OF AGRICULTURE

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By

NURUL FATIAH BINTI SHABUDIN

A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, 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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ENDORSEMENT

This project entitled "Antibacterial Assay and Molecular Identification of Selected Antagonistic Actinomycete Strain against Bacterial Leaf Blight Disease Pathogen, *Xanthomonas oryzae* pv. *oryzae* " is prepared by Nurul Fatiah Binti Shabudin and submitted to Faculty of Agriculture in fulfilment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

Student's name — Student's signature

Nurul Fatiah Binti Shabudin ……

Certified by:

……..

Supervisor's signature

Associate Professor Dr. Kamaruzaman Sijam

Department of Plant Protection

Date:

ABSTRACT

Bacterial Leaf Blight (BLB) caused by *Xanthomonas oryzae pv. oryzae* is one of the most destructive disease affecting rice production worldwide with yield losses recorded up to 60% in Asia. Current control methods are now focusing on chemical pesticides implementation which lead to environmental pollution and increase human health awareness. Therefore, microbe-based biocontrol agent such as selected actinomycetes strain and other antagonistic microbe can be used as an alternative and safer solution to control BLB disease. Actinomycetes strain have been reported to produce larger number of bioactive compound which effective against important plant pathogenic bacteria and fungi. The aims of this research, 1) to obtain antibacterial crude extract using different extraction methods which are broth, activated charcoal and agar and 2) to identify actinomycete strain using molecular method via 16s rRNA. Through this experiment, only broth and activated charcoal extraction method were able to show an antibacterial activity against *Xoo* pathogen. For broth extraction method, only ethyl acetate solvent showed a 10 mm inhibition zone on the MHA agar at 7 days inoculation. Meanwhile, for activated charcoal extraction method, only ethyl acetate solvent managed to show an antibacterial activity for 7 days, 14 days, and 21 days with 6 mm, 15 mm, and 13 mm inhibition zone respectively. Molecular identification via 16s rRNA amplification revealed that selected actinomyces strain belonged to the genus *Streptomyces*.

ABSTRAK

Penyakit hawar daun padi yang disebabkan oleh *Xanthomonas oryzae* pv. *oryzae* adalah salah satu penyakit yang paling menjejaskan pengeluaran beras di seluruh dunia dengan kerugian hasil sehingga 60% di Asia. Kaedah kawalan sekarang yang tertumpu kepada racun kimia membawa kepada pencemaran alam sekitar dan meningkatkan kesedaran kesihatan manusia. Oleh itu, berdasarkan ejen kawalan biologi seperti aktinomiset dipilih sebagai alternatif dan penyelesaian yang lebih selamat untuk mengawal penyakit hawar daun padi ini. Aktinomiset telah dilaporkan untuk menghasilkan jumlah sebatian bioaktif yang berkesan terhadap penyakit tumbuhan dan kulat. Tujuan kajian ini, 1) untuk mendapatkan ekstrak mentah antibakteria menggunakan kaedah pengekstrakan yang berbeza iaitu larutan, arang aktif dan agar dan 2) Untuk mengenal pasti aktinomiset menggunakan kaedah molekul iaitu 16s rRNA . Melalui eksperimen ini, hanya kaedah pengekstrakan daripada larutan arang aktif dan mampu menunjukkan aktiviti antibakteria terhadap *Xoo* patogen. Bagi kaedah pengekstrakan larutan, hanya pelarut etil asetat menunjukkan zon perencatan 10 mm pada agar MHA pada 7 hari inokulasi. Sementara itu, bagi kaedah pengekstrakan arang aktif, hanya pelarut etil asetat berjaya menunjukkan aktiviti antibakteria selama 7 hari, 14 hari dan 21 hari dengan masing-masing 6 mm, 15 mm, dan zon perencatan 13 mm. Pengesahan molekul melalui 16s rRNA mendedahkan bahawa *actinomyces* dipilih tergolong dalam genus *Streptomyces*.

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ABBREVIATIONS

µL	Micro Liter
BLAST	Basic Local Alignment Search Tool
BLB	Bacterial leaf blight
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
MHA	Mueller Hilton
mL	Mili Liter
mm	Mili Meter
^{o}C	Degree Celsius
PCR	Polymerase Chain Reaction
PSA	Peptone Sucrose Agar
rDNA	Ribosomal DNA
rpm	Round per Minute
Xoo	*Xanthomonas oryzae* pv. *oryzae*
YM	Yeast Malt

CHAPTER 1

INTRODUCTION

Rice or *Oryza sativa* L. is a staple food crop for the world population including Malaysia. The rapid growth of human population and increasing in incomes influences the rising of consumer demand. In recent years, most countries facing insufficient problem of rice production. The self-sufficiency level of rice in Malaysia is at 73%; however, our target self-sufficiency level of rice is at 86% by 2010 (MARDI, 2016). The available resources probably cannot support the demand from consumers and later on lead to scarcity of food. Therefore, in order to meet consumer demand and nutritional needs, agriculture should be able to produce high yield and obey the sustainable agriculture principles, which are safe to public health, environment and social well-being (Hanson et al., 2007).

One of the factors that lead to insufficient level of rice is disease. Rice diseases have always had a significant impact on rice supply (Dordas., 2008). Rice cultivation received threat from almost 20 different microbial diseases including Bacterial Leaf Blight (BLB) disease which shows the most devastating and fatal outcome (IRRI, 1994).

The causal bacterium of BLB disease is *Xanthomonas oryzae pv oryzae* (*Xoo*). The severity of BLB disease affecting rice production worldwide with yield losses recorded can go up to 60% in Asia (Adhikari et al., 1994; Dinh et al., 2008; Gnanamanickam, 2009). Development of strategies is required to control or if possible, eliminate the BLB disease threat in rice cultivation area to avert an epidemic.

However, current control methods are now focusing on chemical pesticides implementation and its lead to environmental pollution and increase human health awareness (Chithrashree et

al., 2011; Ndonde and Semu., 2000). Thus, researchers attempt to pursue with more economical and environmental friendly methods such as biological control. The use of microorganisms as biocontrol agent to control *Xoo* pathogen has shown significant potential in being an ecologically-conscious and cost-effective solution in rice cultivation (Gnanamanickam, 2009; Park et al., 2011).

Actinomycetes is a fungal-like microorganisms which have potential to control *Xoo* pathogen. Actinomycetes especially from *Streptomyces* genus, were proven to produce various bioactive compounds including antibiotics (Anzai et al., 2008). *Streptomyces* can be utilized as a bacterial antagonist against *Xoo* pathogen. Research conducted by Hop et al. (2014) has confirmed that actinomycetes strains especially *Streptomycetes toxytricini* were able to inhibit the growth of *Xoo* races.

In the present study, crude extract of selected actinomycetes strain which isolated from previous research was extracted by several solvents (ethyl acetate, chloroform, and hexane) and were screened for antibacterial activity against *Xoo* pathogen. Actinomycete were identified through molecular method. This study was undertaken with following objectives:

1. To obtain antibacterial crude extract using different extraction methods
2. To identify actinomycete strain using molecular method.

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