UNIVERSITI PUTRA MALAYSIA

SCREENING OF ANTIMICROBIAL ACTIVITY OF BACTERIA FROM NATURAL HABITATS TO CONTROL *Xanthomonas campestris pv. campestris* IN CABBAGE

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FP 2017 78

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SERDANG, SELANGOR

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SCREENING OF ANTIMICROBIAL ACTIVITY OF BACTERIA FROM NATURAL HABITATS TO CONTROL *Xanthomonas campestris pv. campestris* IN CABBAGE

BY

LIU JIAN

A project report submitted to Faculty of Agriculture,

Universiti Putra Malaysia,

In fulfillment of the requirement of PRT4999 (Final Year Project) for the award of the degree in Bachelor of Agriculture Science

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SERDANG, SELANGOR DARUL EHSAN

2017/2018

CERTIFICATION

This project report entitled "Screening of Antimicrobial Activity of Bacteria from Natural Habitats to control Xanthomonas campestris pv campestris in cabbage" is prepared by Liu Jian and submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT4999(Final Year Project) for the award of the degree in Bachelor of Agriculture Science.

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ACKNOWLEDGEMENTS

I would like to express my gratitude to all those who helped me during the writing of this thesis.

Firstly, I would like to dedicate grateful appreciation to Dr. Tan Geok Hun. She has walked me through all the stages of the writing of this thesis. Without her consistent and illuminating instruction, this thesis could not have reached its present form.

Secondly, I would like to express my gratitude to the assistant Miss. Musliayana Mansor and Miss. Nor Umaira Abdul Asan. Microbial Culture Collection Unit (UNICC), Institute of Bioscience (UPM). I am also extremely grateful to all my friends and classmates who have kindly provided me assistance and companionship in the course of preparing this paper. In addition, many thanks go to my family for their unfailing love and unwavering support.

Finally, I am really grateful to all those who devote much time to reading this thesis and give me much advice, which will benefit me in my later study.

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LIST OF ABBREVIATIONS

NA	Nutrient Agar
NB	Nutrient Broth
No	Number
℃	Degree Celsius
CFU	Colony Forming Unit
cfu/ml	Colony Forming Unit Per Milliliter
mm	Milliliter
µl	Microliter
SAS	Statistical Analysis System
ANOVA	Analysis of Variance
AST	Antimicrobial Susceptibility test

ABSTRACT

The black rot disease is a serious disease on *Brassica oleracea* caused by *Xanthomonas campestris pv. campestris*. The majority of cabbages were affected by black rot disease and caused economic losses in Malaysia. The problem I am trying to solve in this study is to use potential bacteria culture to inhibit the *Xanthomonas campestris pv. campestris* growth. The approach which I adopted is antimicrobial susceptibility test (AST). A total of 106 bacteria samples were used for AST. Each sample was repeated twice. Result showed that 32 bacteria have the potential to control *Xanthomonas campestris pv. campestris in vitro*. The data were analyzed with ANOVA and mean separations of LSD tests using SAS 9.4. Sample No.12, No.15, No. 24 and No.28 showed bigger size inhibition areas as compared to control. These four samples were also characterized for gram staining and identified using 16s rRNA molecular method. From identification result, No.12 was identified as *Bacillus cereus*; No.15 as *Brevibacillus parabrevis*. Bacteria No.24 and No.28 were identified as *Bacillus subtilis.*

ABSTRAK

Penyakit reput hitam adalah penyakit yang serius pada *Brassica oleracea* disebabkan oleh *Xanthomonas campestris pv. campestris.* Majoriti kubis akan terjejas oleh penyakit reput hitam dan menyebabkan kerugian ekonomi di Malaysia. Kajian ini dijalankan untuk melawan *Xanthomonas campestris pv. campestris* dengan menggunakan bakteria bermanfaat. Pendekatan yang saya gunakan untuk menyelesaikan masalah ini adalah dengan aktiviti antimikrob (AST) bakteria untuk mengawal *Xanthomonas campestris pv. campestris* dalam kubis sebanyak. 106 sampel bakteria sampel telah digunakan untuk ujian aktiviti antimikrob. Setiap sampel diulangi 2 kali. Keputusan menunjukka 32 bakteria yang boleh mengawal *Xanthomonas campestris pv. campestris*. Semua data dianalisis dengan ANOVA dan pemisahan min ujian LSD menggunakan SAS 9.4. Sampel No.12, No.15, No.24 dan No.28 menunjukkan zon cerah yang besar dibandingkan dengan kawalan. Empat sampel tersebut juga dikaji dengan pewarnaan gram dan dikenalpasti dengan menggunakan cara molekular. Keputusan menunjukkan, No. 12 ialah *Bacillus cereus*; No.15 ialah *Brevibacillus parabrevis* manakala, No.24 ialah *Bacillus subtilis* dan No.28 ialah *Bacillus subtilis*.

CHAPTER 1

INTRODUCTION

The *Xanthomonas* belong to the gram-negative bacteria and cause many of diseases in plants. For example, *X. campestris pv. vesicatoria* caused bacterial spot disease in pepper and tomato plants (Thieme et al., 2005); *X. oryzae pv. oryzicola* caused bacterial leaf streak in rice (Bogda- nove et al., 2011); *X. oryzae pv. oryzae* caused bacterial blight disease in rice (Lee et al., 2005; Salzberg et al., 2008) and *X. campestris pv. campestris* caused black rot disease in crucifers. (da Silva *et al*., 2002; Qian et al., 2005; Vorholter et al., 2008). The *X. campestris pv. campestris* causes black rot disease on crucifers in the world, including cabbage, spinach, mustard, cauliflower, kale, broccoli, carrot etc. In additional, it can cause damage on Gui-xiang bamboo, violet and other flowers. The *X. campestris pv. campestris* can cause disease on 10 different vegetables from crucifers, but the cucumber is not significant on this disease. The *X. campestris pv. campestris* is a serious disease on *Brassica oleracea*. The majority of cabbages will be affected by this disease and causes economic losses. The *X. campestris pv. campestris* is the causal agent of black rot disease of crucifer. This disease is very popular on the cabbages in low latitudes areas (Williams, 1980). The bacterium can intrude into the soil and cabbage seed. The black rot disease have quite infected on cabbages during the warm and humid-seasons in the part of counties(Massomo et al., 2004). The disease was widespread in the United States in 1973s, causing serious economic losses of 60% in Malaysia in the 1970s until to 20th

century for more than 80 years.

The pathogens growth on the hydathode regions and causes some of the symptoms in cabbage. The black rot disease causes yield loss of cabbages was achieved at 100% in Tanzania during El-Niño rains in 1998 (Massomo, 2002). The symptoms of black rot are V-shaped lesions and black veins on the leaf. The black rot disease will infection the root of plant through the margin of vascular tissue of the petiole of cabbage(Assis et al., 1999). The bacteria will growth fast when the temperature is excess of 25 ℃ such as Malaysia. Thailand and Myanmar etc. the black rot can be observed after bacteria infection the plants after 10-15days (Williams, 1980).

The majority of smallholder farmers use chemical methods to control the black rot disease. It is necessary to seek amount of methods to control the disease in the development countries. In recent years, there are a lot of methods to control this disease such as physical, chemical, cultural practices. Such as certified seeds, hot-water treatment of seeds , insect ,crop rotation and fungicide (Assis et al., 1999). However, some of the methods is ineffective to control the disease because of the crops can be resistant to the chemical ingredient when farmer apply a lot of chemical pesticides. The chemical pesticides contain some toxic substances which are released into soil, air and underground water to pollute our environmental. Nowadays, it is better to use microbial antagonists from natural habitats to control *X. campestris pv.*

campestris in cabbage. The microbial antagonists are most beneficial method for our environment.

The black rot will cause the low yield, unsustainable production and economic loss of farmers in Malaysia. In this study, 35 types of bacteria were tested against *X. campestris pv. campestris* which caused disease in cabbage in Malaysia. This project was conducted in Microbial Culture Collection Unit (UNICC), Institute of Bioscience, Universiti Putra Malaysia (UPM).

Objective

The specific objective of this research is to select potential bacteria from natural habitats to control *X. campestris pv. campestris* on cabbage based on antimicrobial susceptibility test (AST).

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