



UNIVERSITI PUTRA MALAYSIA

**ENDOPHYTIC MICROORGANISMS FOR IMPROVEMENT OF
BANANA VIGOUR AND TOLERANCE TO FUSARIUM WILT**

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FP 2005 9



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By

ADELINE TING SU YIEN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

October 2005



DEDICATIONS

**I dedicate the fruits of my labour
to my loving late Mum,
to my understanding family,
and my affectionate husband Steve.
Thank you for believing in me.**



Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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Chairperson: Professor Sariah Meon, PhD

Faculty : Agriculture

This study explored the potential of endophytic microorganisms (EMs) isolated from wild bananas as biocontrol agents (BCAs) against Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* race 4 (FocR4) in susceptible banana ramets (Berangan cv. Intan).

The fungal (*Fusarium oxysporum* (UPM31P1)) and bacterial endophytes (*Serratia marcescens* (UPM39B3)) were established as effective BCAs; improving growth and vigour and enhancing tolerance of susceptible ramets to Fusarium wilt. They were antagonistic towards FocR4, with Percentage Inhibition of Radial Growth (PIRG) values of 65% and 63%, respectively. Inhibitory substances were produced in the form of volatile as well as non-volatile substances. The endophytes were also able to colonize the host tissues,



including the roots and corms in artificial inoculation under laboratory conditions. The association of *F. oxysporum* (UPM31P1) and *S. marcescens* (UPM39B3) with the host plants resulted in enhanced vegetative growth as shown by the increase in height, pseudostem diameter, root mass and total number of leaves ramet¹. Endophytes acted as elicitors in the production of inducible compounds associated with induced resistance (peroxidase, polyphenoloxidase, phenylalanine ammonia lyase, total soluble phenol and lignin content). The robust growth and occurrence of induced resistance subsequently enhanced tolerance of the ramets to Fusarium wilt based on parameters such as delay in onset of symptoms, lower percentages in disease incidence, disease severity, and epidemic rate. Inoculation with *F. oxysporum* singly was most effective followed by inoculation with mixture with *S. marcescens* (UPM31P1+UPM39B3). However, the survival and subsequent biocontrol efficacy of *F. oxysporum* (UPM31P1) and *S. marcescens* (UPM39B3) might be vulnerable to the changing soil and environmental conditions. Populations of endophytes were not sustained over time. Therefore, further studies regarding formulation and application frequency and techniques, are essential to maximize the potential of *F. oxysporum* (UPM31P1) and *S. marcescens* (UPM39B3) as BCAs against Fusarium wilt of banana.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**MIKROORGANISMA ENDOFIT UNTUK MENINGKATKAN TUMBESARAN
DAN TOLERANSI TANAMAN PISANG TERHADAP
PENYAKIT LAYU FUSARIUM**

Oleh

ADELINE TING SU YIEN

Oktober 2005

Pengerusi : Profesor Sariah Meon, PhD

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Kajian ini mengeksplorasi potensi mikroorganisma endofit (EMs) yang diasingkan dari pokok pisang liar sebagai agen kawalan biologi (BCAs) untuk menangani penyakit layu Fusarium yang disebabkan oleh *Fusarium oxysporum* f. sp. *cubense* ras 4 (FocR4) pada ramet pisang (Berangan cv. Intan).

Isolat kulat *Fusarium oxysporum* (UPM31P1) dan bakteria *Serratia marcescens* (UPM39B3) dikenalpasti sebagai agen kawalan biologi yang efektif; meningkatkan tumbesaran dan toleransi ramet pisang terhadap layu Fusarium. Kedua-dua isolat ini adalah antagonistik terhadap FocR4, masing-masing dengan peratus perencatan pertumbuhan miselium (PIRG) 65% dan 63%. Bahan perencatan dikeluarkan dalam bentuk bahan mudah meruap dan tidak mudah meruap. Kedua-dua endofit ini juga berupaya menjajah tisu perumah, termasuk

tisu akar dan umbisi, melalui kaedah inokulasi tiruan. Gabungan *F. oxysporum* (UPM31P1) dan *S. marcescens* (UPM39B3) dengan perumah menghasilkan peningkatan pada tumbesaran vegetatif seperti yang ditunjukkan oleh peningkatan dalam ketinggian, lilitan pseudostem, massa akar dan jumlah bilangan daun ramet¹. Endofit bertindak sebagai elisitor dalam pengeluaran kompaun teraruh berkaitan dengan koresistanan teraruh (peroksidase, polifenoloksidase, fenelalanin ammonia liase, fenol terlarut dan kandungan lignin). Peningkatan tumbesaran dan pengeluaran kompaun teraruh meningkatkan ketahanan ramet pisang terhadap layu Fusarium berdasarkan kepada parameter seperti pelambatan kemunculan simptom, pengurangan peratus insiden dan keterukan penyakit dan kadar epidemik. Penginokulatan dengan *F. oxysporum* (UPM31P1) sahaja adalah paling efektif, diikuti dengan inokulasi secara kombinasi dengan *S. marcescens* (UPM31P1+UPM39B3). Namun demikian, kemandirian dan keberkesanan kawalan biologi *F. oxysporum* (UPM31P1) dan *S. marcescens* (UPM39B3) mungkin dipengaruhi oleh keadaan tanah dan alam sekitar yang tidak menentu. Populasi kedua-dua isolat endofit juga menurun mengikut masa. Oleh itu, kajian seterusnya mengenai formulasi dan frekuensi dan teknik aplikasi adalah penting untuk memaksimumkan potensi *F. oxysporum* (UPM31P1) dan *S. marcescens* (UPM39B3) sebagai agen kawalan biologi untuk layu Fusarium pada pisang.



ACKNOWLEDGEMENTS

I thank God for His grace and blessings, in the many moments of my life. Praise be to God for His sovereign love, mercy and faithfulness.

I extend my gratitude to Professor Dr. Sariah Meon, for her constant guidance and constructive criticisms throughout the supervision of the project. Also for her kind and sharing gesture for the many opportunities for scientific interaction availed to me. My heartfelt appreciation as well to Associate Professor Dr. Jugah Kadir, Professor Dr. Son Radu and Dr. Gurmit Singh, for their guidance and suggestions to continuously improve the project. Truly, I am exceedingly grateful for their commitments to this project.

My sincerest appreciation as well to the Malaysian Ministry of Science, Technology and Innovation (MOSTI) for the National Science Fellowship (NSF) awarded. I am also most grateful to the assistance provided by Madam Ho Yuk Wah and her team in United Plantations Bhd (Teluk Intan), not just during the course of my field-trial but the many times involved in preparations of plant materials. Many thanks as well to the staffs and officers from the Plant Pathology Laboratory and Plant Protection Department for their kind assistance.



And, finally, to my family and husband, I thank you for your love, patience, understanding and support, which has kept me accompanied through the high and low points throughout the period of my study. Especially to Steve, thank you for absorbing the weight of my anxieties so I shall not stand alone.



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LIST OF ABBREVIATIONS

ANOVA	Analysis Of Variance
AP-PCR	Arbitrarily Primed-Polymerase Chain Reaction
AUDPC	Area Under Disease Progress Curve
BCA	Biological Control Agent
bp	Base Pair
BUG	Biolog Universal Media
c.f.u.	Colony Forming Units
CRD	Complete Randomized Design
DAF	DNA Amplification Fingerprinting
DI	Disease Incidence
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribonucleoside Triphosphates
DOA	Department of Agriculture
DR	Disease Reduction
DS	Disease Severity
EDTA	Ethylenediaminetetraacetic Acid
EMBRAPA	Brazilian Agricultural Research Cooperation
EMs	Endophytic Microorganisms
FAA	Formalin Acetic Acid
FAO	Food and Agriculture Organization
FHIA	Honduras Foundation of Agricultural Research

