UNIVERSITI PUTRA MALAYSIA

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

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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 ramet^{-1}. 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

Fakulti : Pertanian

Kajian ini mengeksploitasi 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 ramet^{-1}. Endofit bertindak sebagai elisitor dalam pengeluaran kompaun teraruh berkaitan dengan keresistanan 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 memaksimakan potensi *F. oxysporum* (UPM31P1) dan *S. marcescens* (UPM39B3) sebagai agen kawalan biologi untuk layu Fusarium pada pisang.

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I certify that an Examination Committee met on 13th October 2005 to conduct the final examination of Adeline Ting Su Yien on her Doctor of Philosophy thesis entitled "Endophytic Microorganisms for Improvement of Banana Vigour and Tolerance to Fusarium Wilt" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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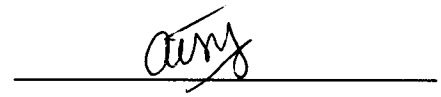
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



ADELINE TING SU YIEN

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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	Brazillian Agricultural Research Cooperation
EMs	Endophytic Microorganisms
FAA	Formalin Acetic Acid
FAO	Food and Agriculture Organization
FHIA	Honduras Foundation of Agricultural Research