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

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

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

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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.
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 FUSARIIUM

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
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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.

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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.
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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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

Date: 12.12.2005
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6.5 Plants from TA (UPM31P1) with no foliar symptoms (A) as compared to plants from TC (control) with yellowish streaks on the leaves (B) and reddish streaks in the pseudostem (C) and corm (D) tissues. (Plants were eight weeks in the field)

6.6 Development of foliar associated symptoms on plants with TA (UPM31P1) (A, B), TB (UPM31P1 + UPM39B3) (C, D) and TC (control) (E, F) at 12 weeks in the field (‘hot-spot’)

6.7 Development of foliar associated symptoms on plants with TA (UPM31P1) (A, B), TB (UPM31P1 + UPM39B3) (C, D) and TC (control) (E, F) at 16 weeks in the field (‘hot-spot’)

6.8 Development of foliar associated symptoms on plants with TA (UPM31P1) (A, B), TB (UPM31P1 + UPM39B3) (C, D) and TC (control) (E, F) at 20 weeks in the field (‘hot-spot’)

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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