



**UNIVERSITI PUTRA MALAYSIA**

***ROOT COLONIZATION AND INDUCTION OF PATHOGENESIS-RELATED  
GENES BY PSEUDOMONAS AERUGINOSA STRAIN UPMP3  
IN OIL PALM***

**SATHYAPRIYA A/P HAMID**

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IN OIL PALM**

**SATHYAPRIYA A/P HAMID**

**MASTER OF SCIENCE**

**UNIVERSITI PUTRA MALAYSIA**

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## DEDICATION

Special dedication to:

*My beloved parents, Mr. and Mrs. Hamid Malliga*

*sister, Ms. Devisri*

*and*

*brothers, Mr. Suriyaraj, Mr. Sathis Kumar and Mr. Heayma Raj*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**ROOT COLONIZATION AND INDUCTION OF PATHOGENESIS-RELATED GENES BY *PSEUDOMONAS AERUGINOSA* STRAIN UPMP3 IN OIL PALM**

by

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**February 2012**

**Chairman: Wong Mui Yun, PhD**

**Institute: Institute of Tropical Agriculture**

Basal stem rot (BSR) disease caused by *Ganoderma boninense* is the most destructive disease in oil palm plantations. The existing control measures for BSR disease such as mechanical, chemical and cultural practices have not been proven satisfactorily. Hence, BSR disease control is preferably achieved within the host plant through induction of resistance. Disease resistance induced by endophytes is effective under field conditions and offers a natural mechanism for biological control of plant disease. The efficient root colonization, proliferation *in situ* and persistence *in planta* have been emphasized on the selection of endophytes in disease control. To date, no study on colonization pattern of endophytic bacteria and endophytic bacteria-induced disease resistance has been reported in oil palm. Thus, the objectives of this study were (i) to tag the selected endophytic bacteria with  $\beta$ -*glucuronidase* gene and green fluorescent protein to facilitate oil palm root

colonization study by selected endophytic bacteria, (ii) to study the root colonization pattern of selected endophytic bacteria and (iii) to detect pathogenesis-related (PR) genes induced by selected endophytic bacteria in oil palm. Basic Local Alignment Search Tool (BLAST) analysis of *recA* gene sequence from *Pseudomonas aeruginosa* strain UPMP3 showed that *P. aeruginosa* strain UPMP3 shared 99% similarity with the clinical strain, *P. aeruginosa* PAO1. Similarly, *Burkholderia cepacia* strain UPMB3 had 99% similarity with *B. cepacia* strain LMG 14087 and *B. cepacia* strain ATCC17759, strains belonging to genovomar I, which is related to non-clinical sources. On the other hand, the absence of *gusA* gene in both, *P. aeruginosa* strain UPMP3 and *B. cepacia* strain UPMB3 demonstrated that tagging these strains with *gusA* was necessary in order to study their root colonization patterns in oil palm roots. For *P. aeruginosa* strain UPMP3, the bacterial cells treated with sterile double distilled water and 10% (v/v) glycerol following electroporation at the field strength 18 kV/cm resulted in transformation efficiency of  $ca 1 \times 10^7$  transformants/ $\mu$ g DNA. Meanwhile, gene transfer in *B. cepacia* strain UPMB3 was done via biparental mating and resulted in transconjugation efficiency of  $ca 1 \times 10^4$  transconjugants/donor CFU. However, tagged *B. cepacia* strain UPMB3 was not selected for further study due to plasmid instability. As the preliminary study of endophytic root colonization by tagged *P. aeruginosa* strain UPMP3 (stated as *P. aeruginosa* strain UPMP3::pHRGFPGUS) in 14 days showed promising results, the subsequent experiment was done with a more thorough study of colonization over 28 days. For epiphytic colonization, the rate increased from 5.76  $\log_{10}$  CFU  $g^{-1}$  FW to 8.19  $\log_{10}$  CFU  $g^{-1}$  FW while the endophytic colonization increased from 4.10  $\log_{10}$  CFU  $g^{-1}$  FW to 6.23  $\log_{10}$  CFU  $g^{-1}$  FW, over 28 days. Confocal laser scanning microscopic analysis of oil palm roots treated with treated

*P. aeruginosa* strain UPMP3::pHRGFPGUS showed that this strain colonized the root elongation zones and lateral root emergence sites after inoculation. Following its ingress, *P. aeruginosa* strain UPMP3::pHRGFPGUS progressed from rhizodermis to exodermis and subsequently to cortical cells intercellularly. Then, the progression continued to the endodermis and finally the xylem vessels and pith. Besides, this strain was shown to associate itself with the cortical cells and vascular tissues of oil palm roots. Induction of pathogenesis-related genes, *chitinase* and  $\beta$ -1, 3 *glucanase* by *P. aeruginosa* strain UPMP3 was studied in oil palm roots in the absence of pathogen. *Chitinase* and  $\beta$ -1, 3 *glucanase* were induced with increasing period after inoculation and showed a peak value at 5 days after inoculation (DAI) and 7 DAI, respectively. The efficacy of *P. aeruginosa* strain UPMP3 in controlling BSR in oil palm seedlings was further screened in the glasshouse. When tested on oil palm seedlings inoculated with *Ganoderma boninense* PER71, *P. aeruginosa* strain UPMP3 suppressed *G. boninense* PER71 compared to the control with disease reduction of 78.36%. The excellent root colonization of *P. aeruginosa* strain UPMP3 coupled with the activation of defence mechanism in oil palm suggest that this strain could be used as the biocontrol agent against *G. boninense*. However, the use of *P. aeruginosa* strain UPMP3 as the biocontrol agent in agriculture has to be strictly monitored due to its potential in causing opportunistic infections in humans.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KOLONISASI AKAR DAN PENCETUSAN GEN YANG BERKAITAN  
DENGAN PATOGENESIS OLEH *PSEUDOMONAS AERUGINOSA* STRAIN  
UPMP3 PADA KELAPA SAWIT**

oleh

**SATHYAPRIYA A/P HAMID**

**Februari 2012**

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Penyakit Reput Pangkal (BSR) yang disebabkan oleh *Ganoderma boninense* merupakan penyakit yang paling merbahaya di ladang kelapa sawit. Langkah pengawalan penyakit menggunakan kaedah sedia ada seperti amalan mekanikal, kimia dan kultur terbukti tidak memuaskan. Oleh itu, kawalan penyakit ini sebaiknya dicapai melalui pencetusan rintangan perumah. Rintangan penyakit yang dicetus oleh endofit adalah berkesan dan menawarkan mekanisme semula jadi untuk kawalan biologi penyakit pada tumbuhan. Pengkolonian akar yang cekap, pembiakan 'in situ' dan pengekalan dalam tumbuhan merupakan aspek yang ditekankan dalam pemilihan endofit pengawal penyakit. Sehingga kini, tiada kajian dilaporkan mengenai corak kolonisasi bakteria endofit dan rintangan penyakit yang dicetuskan oleh bakteria endofit pada pokok kelapa sawit. Oleh itu, objektif kajian ini adalah (i) untuk menanda bakteria endofit terpilih dengan gen ' $\beta$ -glucuronidase' dan 'green

fluorescent protein' bagi memudahkan kajian pengkolonian akar kelapa sawit oleh bakteria endofit terpilih, (ii) untuk mengkaji corak kolonisasi akar oleh bakteria endofit terpilih dan (iii) untuk mengesan beberapa gen yang berkaitan dengan patogenesis yang dicetus oleh bakteria endofit terpilih pada kelapa sawit. Analisis 'Basic Local Alignment Search Tool' (BLAST) ke atas nukleotid gen *recA* daripada *P. aeruginosa* 'strain' UPMP3 telah menunjukkan bahawa *P. aeruginosa* 'strain' UPMP3 mempunyai persamaan sebanyak 99% dengan 'strain' klinikal iaitu *P. aeruginosa* PAO1. *Burkholderia cepacia* strain UPMB3 pula mempunyai 99% persamaan dengan *B. cepacia* strain LMG 14087 dan *B. cepacia* strain ATCC17759, iaitu 'strain' yang tergolong dalam genovomar I, di mana ia selalu dikaitkan dengan sumber tidak klinikal. Ketidakhadiran *gusA* dalam *P. aeruginosa* 'strain' UPMP3 dan *B. cepacia* 'strain' UPMB3 menunjukkan bahawa kedua-dua bakteria harus ditanda dengan *gusA* untuk mengkaji corak kolonisasi akar pokok kelapa sawit. Bagi *P. aeruginosa* 'strain' UPMP3, sel-sel bakteria yang telah dirawat dengan air suling steril dwipenyulingan dan 10% (v/v) gliserol diikuti dengan elektroporasi pada kuasa bidang 18 kV/cm telah menghasilkan kecekapan transformasi sebanyak  $1 \times 10^7$  'transformants'/ $\mu\text{g}$  DNA. Sementara itu, pemindahan gen dalam *B. cepacia* 'strain' UPMB3 telah dilakukan melalui pengawanan dua induk, di mana ia telah menghasilkan kecekapan konjugasi silang sebanyak  $1 \times 10^4$  'transconjugants'/penderma CFU. Walau bagaimanapun, *B. cepacia* 'strain' UPMB3 tidak dipilih untuk kajian lanjutan berikutan ketidakstabilan plasmid. Oleh kerana kajian awal ke atas kolonisasi akar kelapa sawit oleh *P. aeruginosa* 'strain' UPMP3 yang telah ditanda (dinyatakan sebagai *P. aeruginosa* 'strain' UPMP3:: *pHRGFPGUS*) dalam 14 hari telah menunjukkan keputusan yang meyakinkan, uji kaji yang seterusnya telah dilakukan dengan lebih teliti selama 28 hari. Kadar



kolonisasi epifit telah meningkat daripada 5.76 log<sub>10</sub> CFU g<sup>-1</sup> FW kepada 8.19 log<sub>10</sub> CFU g<sup>-1</sup> FW manakala kolonisasi endofit telah meningkat daripada 4.10 log<sub>10</sub> CFU g<sup>-1</sup> FW kepada 6.23 log<sub>10</sub> CFU g<sup>-1</sup> FW, dalam 28 hari. Analisis imej mikroskop pengimbasan laser 'confocal' menunjukkan bahawa bakteria ini telah mengkoloni zon pemanjangan akar dan tapak kemunculan akar sisi selepas inokulasi. Berikutan kemasukan, *P. aeruginosa* 'strain' UPMP3::*pHRGFPGUS* telah maju dari 'rhizodermis' ke eksodermis dan seterusnya sel kortek secara 'intercellular'. Kemudian, ia maju ke endodermis dan akhirnya tisu vaskular termasuk xilem dan 'pith'. Selain itu, 'strain' ini telah menyekutukan dirinya dengan sel-sel kortikal dan tisu vaskular akar kelapa sawit. Pencetusan gen yang berkaitan dengan patogenesis iaitu *chitinase* dan  $\beta$ -1, 3 *glucanase* di dalam akar kelapa sawit oleh *P. aeruginosa* 'strain' UPMP3 telah dikaji tanpa kehadiran patogen. *Chitinase* dan  $\beta$ -1, 3 *glucanase* telah dicetus dengan masa selepas inokulasi dan menunjukkan nilai puncak pada 5 dan 7 hari selepas inokulasi, masing-masing. Seterusnya, keberkesanan *P. aeruginosa* 'strain' UPMP3 dalam kawalan penyakit reput pangkal pada anak benih kelapa sawit dikaji di rumah kaca. Apabila diuji ke atas anak benih kelapa sawit yang diinokulat dengan *Ganoderma boninense* PER71, *P. aeruginosa* 'strain' UPMP3 telah mengawal penyakit reput pangkal dengan pengurangan penyakit sebanyak 78.36%. Keupayaan untuk mengkoloni akar yang baik serta pengaktifan mekanisme pertahanan dalam kelapa sawit mencadangkan bahawa *P. aeruginosa* 'strain' UPMP3 boleh digunakan sebagai agen kawalan biologi terhadap *G. boninense*. Namun, penggunaan *P. aeruginosa* 'strain' UPMP3 sebagai agen kawalan biologi dalam bidang pertanian hendaklah dipantau rapi kerana ia berpotensi menyebabkan jangkitan pada manusia.

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I certify that an Examination Committee has met on **20<sup>th</sup> February 2012** to conduct the final examination of **Sathyapriya a/p Hamid** on her **Master of Science** thesis entitled “**Root colonization and induction of pathogenesis-related genes by *Pseudomonas aeruginosa* strain UPMP3 in oil palm (*Elaeis guineensis* Jacq.)**” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

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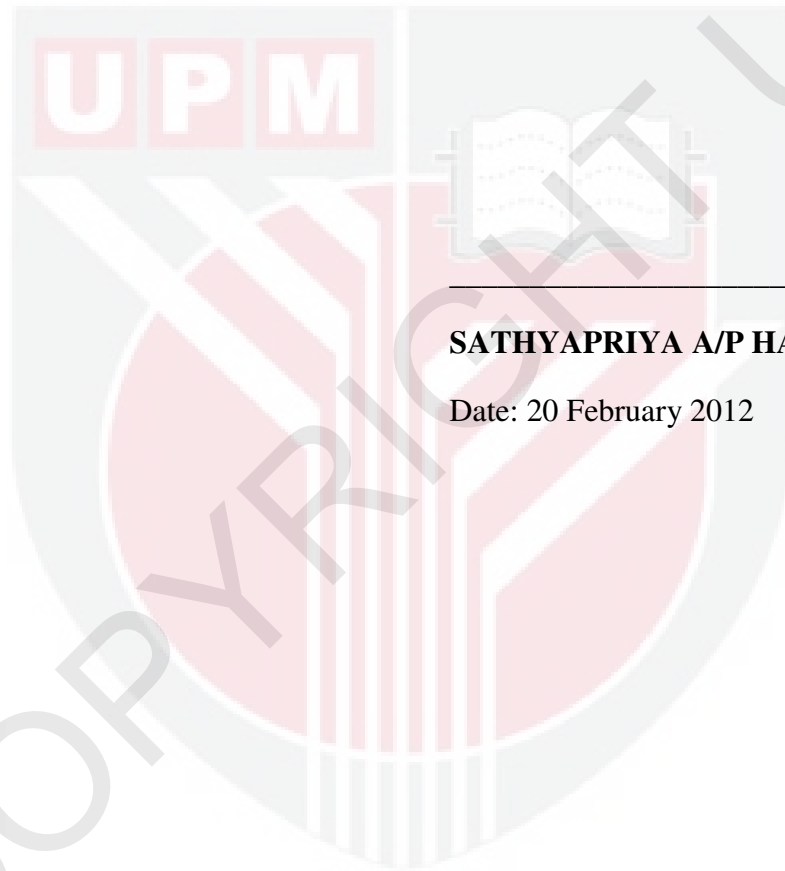
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## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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**SATHYAPRIYA A/P HAMID**

Date: 20 February 2012

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