BACTERIAL ENDOPHYTES FROM OIL PALM (*ELAEIS GUINEENSIS*) AND THEIR ANTAGONISTIC ACTIVITY AGAINST *GANODERMA BONINENSE*

By

ZAITON BINTI SAPAK

Thesis Submitted to the School Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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DEDICATION

Special dedication to:

My parents

They began my education

They motivated me to continue it

They will always contribute to it

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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Chairman : Professor Sariah Meon, PhD

Faculty : Agriculture

The oil palm (*Elaeis guineensis*) is the most important oil crop in Malaysia, producing an` ` average yield of approximately four tonnes of palm oil per hectare per year. In Malaysia, the crop is blessed by being largely disease free, suffering from but one major disease, Basal Stem Rot (BSR), caused by *Ganoderma boninense*. With no known cure at present, it is the major cause of disease in oil palm and therefore of great economic importance to the Malaysian oil palm industry.

Endophytic bacteria have been found able to induce systemic resistance in plants and shown biological traits like antibiotic activity and lysis. The use of

endophytic microorganisms should be preferable to other biological control agents as they are internal colonizers, and therefore more able to compete in the vascular systems, depriving *Ganoderma* from both nutrients and space for its proliferation. The potential of symptomless endophyte colonization for protection against disease has provided great impetus for research. Understanding their endophytic life stages may help in the design of management strategies for controlling BSR. The objectives of this study were, therefore, to isolate, characterize and identify the symptomless bacterial endophytes in oil palm and to determine their potential as biocontrol agents against *Ganoderma in vitro* and *in vivo*.

The bacterial endophytes were detected and isolated from the roots of oil palm on different soils, palm age and BSR infection status. Observations of histological root sections under a light microscope showed the presence of bacterial endophytes, stained red with safranin. The bacterial cells were found both intra- and intercellular in the root tissues and distributed uniformly in the epidermis, sclerenchyma and cortical cells. They were also detected in the vascular tissues around the phloem and xylem vessels in the roots of symptomless palms. However, in the roots of palms with BSR, Gram-negative bacteria were also found randomly in the epidermis, sclerenchyma and cortex, but not in the phloem and xylem vessels. Of 1,323 microbial endophytes isolated from 3,600 oil palm roots, 65.23% were bacteria, 32.73% fungi and 2.04% actinomycetes. 93.04% of the bacteria were Gram-

negative and only 6.95% gram-positive. Contrast comparisons between the frequency of bacterial and fungal isolates for different age palms showed a significant difference with more bacterial and fungal endophytes in mature (≥11 years) followed by middle-aged (6-10 years) and young (1-5 years) palms from all the sampling areas. In comparing the soils, the frequency of endophytic bacteria recovered from symptomless palms was not significantly different between coastal and peat soils. However, all the inland soils had a significantly lower frequency of endophytic bacteria with high abundance of the genera *Pseudomonas* and *Burkholderia*. The fungal abundance was not significantly different between all the areas sampled. From the symptomatic palms, abundant bacteria were found in inland but lower in coastal and peat areas.

The isolated bacteria were screened *in vitro* for their antagonistic properties towards *G. boninense*. Three bacterial endophytes (B3, P3 and S19) were established to have potential as biocontrol agents based on their percentage inhibition of radial growth (PIRG) of 70.80%, 75.95% and 55.63%, respectively. They were also effective host colonizers and could be re-isolated from the roots, 24 hours after inoculation. B3, P3 and S19 were identified by the Biolog® system as *Burkholderia cepacia, Pseudomonas aeruginosa* and *Serratia marcescens* respectively. Based on their colonization, establishment and localization in oil palm roots, *B. cepacia* (B3) and *P. aeruginosa* (P3) were selected for further *in vivo* screening for their

efficacy in controlling BSR in oil palm seedlings in the glasshouse. When tested on oil palm seedlings inoculated with *G. boninense*, both of them, alone and in mixture suppressed *G. boninense* compared to the control. Not only did they delay the onset of BSR symptoms but also promoted the growth of the seedlings. Pre-inoculating the seedlings with *P. aeruginosa* had their BSR incidence reduced by 76.27%, *B. cepacia* reduced the disease incidence by 42.2% and the mixture of *P. aeruginosa* and *B. cepacia* by 54.24%. Based on disease reduction, the oil palm seedlings with *P. aeruginosa* were most effective in suppressing BSR as compared to other treatments.

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

BAKTERIA ENDOFIT DARIPADA KELAPA SAWIT (*ELAEIS GUINEENSIS*) DAN AKTIVITI ANTAGONISTIK MELAWAN *GANODERMA BONINENSE*

By

ZAITON BINTI SAPAK

September 2006

Pengerusi : Profesor Sariah Meon, PhD

Fakulti : Pertanian

Kelapa sawit (*Elaeis guineensis*) adalah tanaman minyak utama di Malaysia, dengan penghasilan purata minyak sebanyak empat tan per hektar per tahun. Di Malaysia, tanaman kelapa sawit bebas daripada kebanyakkan penyakit kecuali penyakit utama yang dikenali sebagai penyakit reput pangkal oleh *Ganoderma boninense*. Sehingga kini tiada penawar ditemui dan ia dianggap sebagai penyebab utama penyakit kepada kelapa sawit dan seterusnya memberi kesan ekonomi terhadap industri kelapa sawit Malaysia.

Bakteria endofit berupaya untuk merangsang sistem pertahanan dalam tumbuhan dan menunjukkan ciri-ciri biologi seperti aktiviti antibiotik dan

lisis. Mikroorganisma endofit lebih sesuai digunakan sebagai agen kawalan biologi kerana ia berada dalam tisu dan dapat bersaing dengan patogen dalam sistem vaskular seperti *Ganoderma*, bagi mendapat nutrien dan ruang. Potensi mikroorganisma endofit untuk melawan penyakit telah memberi peluang yang baik dalam penyelidikan. Memahami kitar hidup endofit mungkin dapat membantu dalam membentuk strategi untuk mengawal BSR. Objektif penyelidikan ini ialah memencil, mengenalpasti komuniti bakteria endofit daripada kelapa sawit dan menentukan potensi mereka sebagai agen kawalan biologi terhadap *Ganoderma* secara *in vitro* dan *in vivo*.

Bakteria endofit telah dikenalpasti dan dipencilkan daripada sampel akar kelapa sawit yang berlainan jenis tanah, umur pokok dan status jangkitan BSR. Pemerhatian secara histologi pada bahagian akar kelapa sawit di bawah mikroskop cahaya telah menunjukkan kehadiran bakteria endofit dengan perwarnaan merah oleh safranin. Sel bakteria didapati berada di dalam dan luar sel tisu akar kelapa sawit dan tersebar secara seragam di dalam epidermis, sel sklerenkima dan kortek. Akar kelapa sawit yang diambil daripada pokok yang sihat juga mempunyai bakteria endofit dalam tisu vaskular termasuk floem dan xilem. Walau bagaimanapun, akar yang diambil daripada pokok yang dijangkiti BSR; bakteria Gram- negatif dijumpai tersebar secara rawak dalam epidermis, sel sklerenkima dan kortek tetapi tidak terdapat dalam floem dan xilem.

Daripada 1,323 mikrob endofit yang telah berjaya dipencilkan daripada 3600 keratan tisu akar kelapa sawit, sebanyak 65.23% adalah bakteria, 32.73% kulat dan 2.04% aktinomisit. 93.04% bakteria adalah Gram-negatif dan hanya 6.95% adalah Gram-positif. Perbandingan secara kontra antara jumlah isolat bakteria dan kulat bagi umur kelapa sawit yang berbeza menunjukkan perbezaan yang bererti dimana bakteria dan kulat endofit lebih banyak terdapat dalam pokok yang berumur matang (≥11 tahun)diikuti umur pertengahan (6-10 tahun) dan muda (1-5 tahun) bagi setiap kawasan persampelan. Perbandingan bagi tanah yang berlainan, frekuensi bakteria endofit yang dipencilkan daripada kelapa sawit yang tidak mempunyai simptom BSR, tidak berbeza antara tanah pantai dan gambut. Walau bagaimanapun, semua kawasan di tanah pendalaman mempunyai frekuensi bakteria endofit yang rendah tetapi mempunyai genus *Pseudomonas* dan *Burkholderia* yang tinggi. Frekuensi kulat tidak menunjukkan perbezaan di semua kawasan persampelan. Kelapa sawit yang mempunyai simptom menunjukkan frekuensi tinggi di kawasan pedalaman tetapi rendah di dalam kelapa sawit dari kawasan tanah pasir dan gambut. Frekuensi kulat tidak menunjukkan perbezaan di semua kawasan persampelan.

Bakteria yang telah diasingkan, disaring secara *in vitro* untuk ciri keantagonisan mereka terhadap *G. boninense*. Tiga bakteria (B3, P3 dan S19) endofit telah dikenalpasti mempunyai potensi sebagai agen kawalan

biologi berdasarkan peratus rencatan pertumbuhan miselium *G. boninense* iaitu masing-masing 70.80%, 75.95% dan 55.63%. Mereka juga berkesan mengkoloni akar kelapa sawit dan dapat dipencil semula selepas 24 jam. B3, P3 dan S19 telah dikenalpasti melalui sistem Biolog® sebagai *Burkholderia cepacia*, *Pseudomonas aeruginosa* dan *Serratia marcescens*. Berdasarkan pengkolonian, kedudukkan mereka dalam tisu akar kelapa sawit, *Burkholderia cepacia* (B3) dan *Pseudomonas aeruginosa* (P3) telah dipilih untuk menilai keupayaan mereka mengawal BSR pada anak benih kelapa sawit di dalam rumah kaca. Apabila diuji pada anak benih kalapa sawit yang diinokulat dengan *G. boninense*, kedua-dua bakteria ini sama ada secara berasingan atau dicampur dapat mengawal *G. boninense* berbanding kawalan. Bukan sahaja dapat melambatkan kemunculan simptom BSR tetapi ia juga dapat meningkat pertumbuhan anak benih kelapa sawit. Anak benih kelapa sawit yang telah inokulat dengan *P. aeruginosa* telah mengurangkan jangkitan BSR sebanyak 76.27%, *B. cepacia* telah mengurangkan jangkitan penyakit sebanyak 42.2% dan campuran *P. aeruginosa* dan *B. cepacia* sebanyak 54.24%. Berdasarkan pengurangan penyakit, anak benih kelapa sawit yang inokulat dengan *P. aeruginosa* lebih berkesan mengawal BSR berbanding rawatan yang lain.

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I certify that an Examination Committee met on 12 September 2006 to conduct the final examination of Zaiton Binti Sapak on her Master of Science thesis entitled " Bacterial Endophytes From Oil Palm (*Elaeis guineensis*) and their antagonistic activity against *Ganoderma boninense*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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The thesis submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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Date: 16 January 2007

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.

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ZAITON BINTI SAPAK

Date: 13 November 2006

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