BIOLOGICAL CONTROL OF SCHIZOPHYLLUM COMMUNE
FR. THE SEEDBORNE PATHOGEN OF OIL PALM WITH
ANTAGONISTIC BACTERIA

By

ANTARJO DIKIN

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

May 2004
DEDICATION

Thanks to Allah SWT for his blessing to my family,

To my wife Ir. Zakiah,

To my son Nicky Rahmana Putra and my daughter Nanda Marizky for their undying support.

To my parents who always pray for me.
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

BIOLOGICAL CONTROL OF SEEDBORNE PATHOGEN OF OIL PALM, SCHIZOPHYLLUM COMMUNE FR. WITH ANTAGONISTIC BACTERIA

By

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March 2004

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Schizophyllum commune Fr. is one of the most important seedborne pathogens of oil palm. This pathogen can be isolated from fermented fruits, germinated seeds, rotten fruits and infected seeds. The fungus produced papery and leathery compact white mycelia on both sides of the agar plates and often produced basidiocarps (diameter less than 2 cm) with gills on culture plates. The mycelia produced clamp connections with some hyphae having spinulose projections. The width of the hyphae was 2 to 4 µ. Spores produced from basidiocarp were hyaline, cylindrical and single-celled. S. commune grew on PDA medium added 50 g/L of NaCl at 30-35°C optimum temperature but the mycelia became dormant at 45°C. The fungus grew on PDA medium at pH 5 to 6 and was resistant to 1% sodium hypochloride.
Inoculation of *S. commune* Fr. on non germinating oil palm seeds by contact was found to cause a significant decrease of seed germination to 64.3%. Mycelia covered the germ pores of seeds and penetrated the germ pore to reach the surface of seed kernel. Inoculation of non germinating seeds produced abnormal seedlings, inhibition of germ tube elongation and brown discoloration of plumule and radicle. Inoculation of germinating seeds resulted in stunted growth of seedlings, decreased root growth and reduction in both fresh and dry weights.

Histological study of the infected seeds indicated that the mycelia penetrated the rotted fruit to reach the testa through germ pores. Mycelium was unable to directly penetrate the endocarp. In advanced seed infection, white mycelia colonized the surface of seed kernel. Mycelia infected the surface of the testa without formation of ‘appressorium’ to support absorption to the surface of the kernel nor produced ‘haustorium’ for absorption of nutrient from the host.

Eight out of 40 bacterial isolates from rotten fruits and infected seeds were found to inhibit the radial growth of *S. commune* in the range 42.9–79.8% and spore germination. The 8 antagonistic bacterial isolates were clustered into 5 species by Biolog® Identification System and they were *Bacillus*
thermoglucosidasius, *Burkholderia cepacia, Pseudomonas aeruginosa, Serratia marcescens* and *Serratia* sp.

*B. cepacia* and *Serratia* sp. grew on NA medium containing 4% and 6% salt concentration respectively. Both species were able to grow on Nutrient Agar (NA) medium that were incubated at 40°C. Both species grew on NA medium containing 0.5% and 2% sodium hypochloride respectively. Both *B. cepacia* and *Serratia* sp. grew on NA medium with pH range 4-8.

Dipped vacuum treatment of antagonistic bacteria at 400 mm Hg vac. for 2 minutes significantly reduced the internal infection of the inoculated non germinating oil palm seeds. *B. cepacia* and *Serratia* sp. significantly increased percentage of seed germination. Dipped vacuum treatment was also used to treat inoculated germinating seeds at 150 mm Hg Vac. for 2 minutes. The results showed that *B. cepacia* reduced seedling infection.
Schizophyllum commune Fr. adalah sejenis kulat penting pada jangkitan biji benih kelapa sawit. Kulat ini boleh diasingkan dari pada buah yang terfermentasi, biji bercambah, buah reput dan jangkitan biji. Kulat ini menghasilkan miselia berwarna putih seperti kertas, dan miselia padat pada permukaan kedua-dua piring petri, ia juga menghasilkan basidiokarpa (diameter kurang dari 2 cm) dengan insang. Miselia mempunyai klem dan berduri. Lebar hifa 2 hingga 4μ. Spora dihasilkan daripada basidiokarpa, putih, silinder dan bersel tunggal. S. commune boleh hidup pada media PDA yang mengandungi NaCl 50 g/L pada suhu optimum 30-35°C, tetapi miselia menjadi dorman pada suhu 45°C. Kulat boleh tumbuh di atas
media PDA pada pH 5 hingga 6 dan tahan hidup pada media PDA yang mengandung natrium hipoklorida 1%.

Inokulasi *S. commune* Fr. pada biji yang belum bercambah dengan kontak telah menyebabkan berkurangan percambahan sebesar 64.3%. Miselia menutupi liang biji dan menembusi hingga ke permukaan endosperma. Inokulasi biji yang belum bercambah menghasilkan percambahan abnormal, menahan pemanjangan saluran percambahan, perubahan warna perang pada plumula dan radikel. Inokulasi pada biji yang telah bercambah menyebabkan percambahan bantut, pertumbuhan akar yang lambat dan berat basah dan berat kering percambahan berkurangan.

Kajian histologi pada biji yang dijangkiti menunjukkan miselia menembusi buah yang reput hingga ke testa melalui liang percambahan. Miselia gagal menembusi endokarpa biji sawit secara langsung. Jangkitan selanjutnya menunjukkan miselia menutupi permukaan endosperma. Miselia menjangkiti permukaan endosperma tanpa pembentukan apresorium untuk menopang plekatan pada permukaan endosperma dan menghasilkan haustorium untuk menyerap makanan dari perumah.

Daripada 40 pemencilan bakteria, lapan daripadanya diperolehi daripada buah yang busuk dan biji benih yang dijangkiti menahan pertumbuhan
miselia pada 42.9-79.8%, percambahan spora dan menghasilkan bahan antikulat. Lapan pemencilan bakteria antagonis dalam kumpulan 5 jenis menggunakan Sistem Identifikasi Biolog® adalah *Bacillus thermoglucosidasius*, *Burkholderia cepacia*, *Pseudomonas aeruginosa*, *Serratia marcescens* dan *Serratia* sp.

*B. cepacia* dan *Serratia* sp. boleh hidup pada media Agar Nutrien (AN) yang mengandung garam klorida 4% dan 6 % berturut-turut. Kedua jenis bakteria boleh hidup pada media AN pada 40°C. Kedua jenis boleh hidup pada media AN yang mengandung natrium hipoklorida 0.5% dan 2% berturut-turut. Kedua jenis boleh hidup pada media AN pada pH 4-8.

Pengawalan rendaman bakteria antagonis tanpa udara pada 400 mm Hg vac. selama 2 minit bermakna mengurangkan jangkitan pada biji benih sawit yang belum bercambah. *B. cepacia* dan *Serratia* sp. bermakna menaikkan peratus percambahan. Rendaman tanpa udara juga boleh mengkawal jangkitan biji benih yang telah bercambah pada 150 mm Hg vac. selama 2 minit. Keputusan *B. cepacia* mengurangkan jangkitan pada anak benih.
ACKNOWLEDGEMENTS

I would like to thank to my advisor, Associate Professor Dr. Kamaruzaman Sijam for his guidance and encouragement throughout the period of my graduate studies. I profusely thank Dr. Zainal Abidin Mior Ahmad for his adviser during the conducting the research as well as critical reading of this manuscript. I am grateful to Dr. Idris B. Abu Seman, Plant Pathology and Weed Science Group, Biological Research Division, Malaysian Palm Oil Board as external advisory committee for his guidance.

I would like to extend my thanks to Dr. Mohammad Zakariah Hussin on his contribution to motivate my work in conducting the research at the first semester as the committee member.

I also gratefully acknowledge Prof. Dr. Sariah Meon for her constructive criticisms during the presentation of research proposal to complete my research on seedborne disease of oil palm.

My sincere gratitude to Guthrie Plantation and Agricultural Service Bhd (especially to Hj. Kamalur Rahman Ibrahim, Mr. Mohd. Nasir B Ngah, Mr. Ismail B Mohamad Yunus) and other staffs of Layang-layang, Johor for providing the highest value of oil palm seeds for conducting the research.
My thankfull extended to Director General of Indonesian Agriculture Quarantine Agency (IAQA), Project Manager of Integrated Pest Management for Small Estate Crop Plantation, Plant Quarantine Component, The Republic of Indonesia to support the finance for Master of Science Programme, in Universiti Putra Malaysia.

Thanks are also extended to the technicians, Mr. Mohd. Yusof Yassin, Mr. Zawawi, Mr. Rozali, Mrs. Junaina, Laboratory of Microbiology, and Mr. Syamsuddin, Laboratory of Nematology, Faculty of Agriculture.

My special thanks to my wife Ir. Zakiah and our children, Nicky Rahmana Putra, and Nanda Marizky, whose patience, understanding and sacrifice make this study possible.

Finally, my greatest appreciation is extended to my father, Haji Mohammad Sodikin, my mother, Almarhuma Mukiyem, and plant quarantine’s friends who studying in UPM, Philippines, Holland, and colleagues of IAQA for their moral support and encouragement during the period of my study in Universiti Putra Malaysia.
I certify that an Examination Committee met on March 30th 2004 to conduct the final examination of Antarjo Dikin on his Master of Science thesis title “Biological Control of Seedborne Pathogen of Oil Palm, Schizophyllum commune Fr. with Antagonistic Bacteria” in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Member 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 currently submitted for any other degree at UPM or other institutions.

_______________________
ANTARJO DIKIN

Date:
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