

ISOLATION AND SCREENING OF RHIZOBACTERIA AND THEIR POTENTIAL
AS PLANT GROWTH PROMOTERS AND BIOLOGICAL CONTROL AGENTS FOR
BACTERIAL WILT OF TOMATO

By

BAMBANG HESTI SUSILO

Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirements for the

Degree of Master of Agricultural Science

September 2004

This work is dedicated

to

my wife, Rosilawati

to

my daughter, Firistyana Silaputri (Ana)

and to

my sons, Adi Saputra (Adi)

and

Thirda Febrilian Putra (Billy)

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

**ISOLATION AND SCREENING OF RHIZOBACTERIA AND THEIR
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September 2004

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Faculty: Agriculture

Isolation of rhizobacteria was carried out from rhizosphere of tomato, eggplant, and groundnut. One hundred and eighty five isolates were obtained and screened for antagonism against *Ralstonia solanacearum*. Out of these, 37 showed antagonistic activity. Five out of the 37 antagonists, namely *Pseudomonas aeruginosa* (Pa I and Pa II), *Pseudomonas sp.* (FA4) and *Serratia marcescens* (Sm R and Sm E), were then chosen for further studies.

Tomato seeds were bio-primed with the five antagonists and seed germination was assessed. Bio-priming of tomato seeds with the antagonist either individually or in combination had no significant effect on seed germination when compared to either the

methylcellulose or distilled water control. Thus, the bio-priming did not give any detrimental effect on seed germination. The use of methylcellulose as binder was also not phytotoxic.

The antagonists were tested for the ability to produce IAA and solubilize phosphate *in vitro*. All antagonists produced IAA at varying concentrations, while only Pa I, Pa II and FA4 were phosphate solubilizers. Evaluation of Pa II, Pa I and Sm E to enhance tomato plant growth was carried out in two greenhouse trials. Firstly, Pa II and Sm E were evaluated based on their production of IAA. Secondly, Pa II and Pa I were evaluated based on their ability to solubilize phosphate. Results from the first trial showed that treatment with Pa II gave significantly ($P \leq 0.01$) higher fresh and dry weight, plant height and root length compared to plants treated with Sm E and the control indicating that the increased growth could possibly be due to the IAA production. In the second trial, the individual treatments with Pa II and Pa I, in a P-deficient soil amended with rock phosphate, gave significantly ($P \leq 0.01$) higher fresh and dry weight, and plant height compared to the control. Results from the second trial indicated that the ability of the isolates to solubilize phosphate could be another mechanism involved in the growth promotion.

Tomato seeds bio-primed with the five antagonists, individually and in combination, followed by application of the antagonists incorporated into rhizosphere using potting-mixture were evaluated for their efficacy in controlling bacterial wilt under greenhouse condition. All treatments significantly ($P \leq 0.01$) reduced severity of the disease and the

disease incidence when compared to the control. The disease incidence was reduced by 83.33% with individual treatments of Sm E, Pa I and Pa II. Combinations of Pa I + Pa II and Sm E + Sm R were able to reduce the disease incidence by 66.67 and 50% respectively, while individual treatment of FA4 and Sm R gave the reduction of 75 and 41.67% respectively.

In conclusion, the five antagonists have the potential as plant growth-promoters and as biological control agents for bacterial wilt of tomato.

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

**PEMENCILAN DAN PENYARINGAN RIZOBAKTERIA DAN
KEUPAYAANNYA SEBAGAI PENGGALAK TUMBESARAN DAN
EJEN KAWALAN BIOLOGI BAGI PENYAKIT LAYU BAKTERIA
PADA TOMATO**

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Pemencilan rizobakteria dijalankan dari rizosfera tomato, terung dan kacang tanah. Seratus lapan puluh lima isolat telah diperolehi dan disaring sifat antagonisnya terhadap *Ralstonia solanacearum*. Daripada jumlah ini, 37 menunjukkan tindak balas antagonis. Lima daripada asingan antagonis ini, *Pseudomonas aeruginosa* (Pa I and Pa II),

Pseudomonas sp. (FA4) dan *Serratia marcescens* (Sm R and Sm E), seterusnya dipilih untuk ujikaji selanjutnya.

Biji benih tomato telah dirawat secara biologi dengan lima antagonis ini dan percambahan biji benih dinilai. Rawatan biologi ke atas biji benih tomato dengan antagonis secara tunggal atau kombinasi tidak memberikan kesan signifikan terhadap percambahan biji benih dibandingkan samada dengan kawalan metilselulosa atau air suling sahaja. Jadi, rawatan biologi didapati tidak memberikan sebarang kesan kerosakan terhadap percambahan biji benih. Penggunaan metilselulosa sebagai pengikat juga tidak memberikan kesan fitotoksik.

Antagonis ini diuji keupayaan menghasilkan IAA dan melarutkan fosfat secara *in vitro*. Semua antagonis menghasilkan IAA pada kepekatan berbeza, manakala hanya Pa I, Pa II dan FA4 adalah pelarut fosfat. Penilaian terhadap Pa II, Pa I dan Sm E untuk meningkatkan pertumbuhan pokok tomato telah dijalankan dalam dua percubaan rumah hijau. Pertama, Pa II dan Sm E dinilai berdasarkan penghasilan IAAnya. Kedua, Pa II dan Pa I dinilai berdasarkan kebolehannya melarutkan fosfat. Keputusan percubaan pertama menunjukkan rawatan dengan Pa II menghasilkan peningkatan yang bererti ($P \leq 0.01$) kepada berat segar dan kering, ketinggian pokok dan pemanjangan akar berbanding dengan pokok yang dirawat dengan Sm E dan kawalan, menunjukkan peningkatan tumbesaran yang berkemungkinan disebabkan oleh penghasilan IAA. Dalam percubaan kedua, rawatan dengan Pa II dan Pa I secara tunggal, dalam tanah kekurangan P yang ditambah dengan batuan fosfat, menunjukkan peningkatan yang bererti ($P \leq 0.01$) pada berat segar dan kering dan ketinggian pokok berbanding kawalan. Keputusan ini

menunjukkan bahawa kebolehan isolat untuk melarutkan fosfat boleh menjadi satu mekanisme dalam peningkatan pertumbuhan pokok.

Biji benih tomato yang dirawat secara biologi dengan lima antagonis, secara tunggal atau kombinasi, diikuti dengan aplikasi penggabungan antagonis ke dalam rizosfera menggunakan “potting-mixture”, dinilai terhadap keberkesanannya mengawal penyakit layu bakteria dalam persekitaran rumah hijau. Keseluruhan rawatan secara bererti ($P \leq 0.01$) mengurangkan keparahan penyakit dan insidens penyakit berbanding kawalan. Insidens penyakit berkurangan sebanyak 83.33% dengan rawatan Sm E, Pa I and Pa II secara tunggal. Kombinasi Pa I + Pa II dan Sm R + Sm E boleh mengurangkan insidens penyakit masing-masing sebanyak 66.67 dan 50%, manakala rawatan FA4 dan Sm R secara tunggal masing-masing mengurangkan 75 dan 41.67%.

Kesimpulannya, kelima-lima antagonis ini berpotensi sebagai rizobakteria penggalak tumbesaran dan sebagai ejen kawalan biologi bagi penyakit layu bakteria pada tomato.

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I certify that an Examination Committee met on 7th September 2004 to conduct the final examination of Bambang Hesti Susilo on his Master of Agricultural Science thesis entitled “Isolation and Screening of Rhizobacteria and Their Potential as Plant Growth Promoters and Biological Control Agents for Bacterial Wilt of Tomato” 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 Examiner 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.

MBANG HESTI SUSILO **BA**

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