UNIVERSITI PUTRA MALAYSIA

APPLICATION OF *BACILLUS SPHAERICUS* UPMB10 FOR GROWTH ENHANCEMENT OF BANANA (*MUSA* SPP. VAR. BERANGAN) AND ITS EFFECT ON FUSARIUM WILT

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By

ILLANI ZURAIHAH BINTI IBRAHIM

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

SEPTEMBER 2008

DEDICATION

Special dedication to:

Zull and Danial

My lovely family…. For all the supports, endless loves and advices throughout the long journey to complete my study…. Keeps me alive!

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

APPLICATION OF *BACILLUS SPHAERICUS* UPMB10 FOR GROWTH ENHANCEMENT OF BANANA (*MUSA* SPP. VAR. BERANGAN) AND ITS EFFECT ON FUSARIUM WILT

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September 2008

Chairman : Professor Zulkifli Hj Shamsuddin, Ph.D.

Faculty : Agriculture

A locally isolated strain *Bacillus sphaericus* UPMB10, categorized as plant growth promoting rhizobacteria (PGPR), was reported to have beneficial effects in stimulating growth and yield of many agricultural crops. This study explores the indirect mechanism of *B. sphaericus* UPMB10 that would support the hypothesis that this PGPR could act as a biocontrol agent. Through *in-vitro* study, *B. sphaericus* UPMB10 was observed to inhibit the growth of *Fusarium oxysporum* f.sp *cubense* race 4 (FOCR4) mycelium by 70% in culture medium. Malformation of the fungal hyphae by vacuole formation, swelling and thickening of the hyphal strands occurred with the presence of *B. sphaericus* UPMB10. In addition, *B. sphaericus* UPMB10 showed positive

results in the tests for production of cell wall-degrading enzymes and phosphate solubilizing activity by producing clear zones or halos. It can also produce a plant growth regulating hormone, indole acetic acid (IAA). An initial glasshouse study showed that inoculation with *B. sphaerius* UPMB10 increased plant growth up to 30% in plant height and increased nutrient uptake namely for N, P, K, and Ca, and also reduced the disease severity through visual symptoms and vascular discoloration of the corm. In the last glasshouse experiment, the appearance of the disease incidence was observed. All of the treatments with FOCR4 application were infected by FOCR4 including those treated with *B. sphaericus* UPMB10. Significantly, the infection occurred at the late stage of the experiment indicating that *B. sphaericus* UPMB10 could delay the infection process. Solid inoculum inoculation with *B. sphaericus* UPMB10 showed a delayed onset of symptoms, lower percentage in disease incidence and epidemic rate. As liquid and solid substrate were selected for evaluation against the disease in the glasshouse, it showed that soil application of *B. sphaericus* UPMB10 with solid inoculum provided the best suppression of Fusarium wilt of banana. In this study, an inoculum dose of 100 g of solid formulation per plant was optimum, because it suppressed the disease to the same extent as higher doses of the antagonist. This showed *B. sphaericus* UPMB10 has a high potential for its use as a plant growth enhancer and biocontrol agent in the disease management of Fusarium wilt in bananas.

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

APLIKASI *BACILLUS SPHAERICUS* UPMB10 UNTUK MENINGKATKAN PERTUMBUHAN PISANG (*MUSA* SPP. VAR. BERANGAN) DAN KESANNYA KE ATAS PENYAKIT LAYU FUSARIUM

Oleh

ILLANI ZURAIHAH IBRAHIM

September 2008

Pengerusi : Professor Zulkifli Hj Shamsuddin, Ph.D.

Fakulti : Pertanian

Strain bakteria tempatan *Bacillus sphaericus* UPMB10 yang dikategorikan sebagai rhizobakteria penggalak pertumbuhan pokok (PGPR) dilaporkan mempunyai keupayaan dalam menggalakkan pertumbuhan dan hasil dalam pelbagai tanaman pertanian. Kajian ini merupakan explorasi yang menunjukkan secara tidak langsung mekanisme yang dapat menyokong hipotesis PGPR ini untuk bertindak sebagai agen kawalan biologi. Melalui kaijan *in vitro*, *B. sphaericus* UPMB10 telah diperhatikan dapat merencatkan pertumbuhan *Fusarium oxysorum* f.sp.*cubense* race 4 (FOCR4) sebanyak 70% di dalam medium kultur. Ketidakseragaman pertumbuhan hifa fungi yang disebabkan oleh pembentukan vakuol, pembengkakkan dan penebalan

jaluran hifa telah berlaku dengan kehadiran *B. sphaericus* UPMB10. Tambahan pula, *B. sphaericus* UPMB10 juga telah menunjukkan keputusan yang positif di dalam ujikaji penghasilan enzim pelupus dinding sel dan ujikaji aktiviti pelarutan fosfat dengan menunjukkan kesan zon jernih yang jelas atau halo. Ia juga berupaya menghasilkan hormon pertumbuhan iaitu indole asetik asid (IAA). Pada permulaan eksperimen rumah kaca menunjukkan yang inokulasi dengan *B. sphaericus* UPMB10 telah meningkatkan pertumbuhan pokok sebanyak 30% ketinggian pokok dan kadar pengambilan nutrient terutamanya N, P, K dan Ca. Ia juga telah mengurangkan kadar keterukan penyakit melalui pemerhatian simptom luaran pokok dan perwarnaan umbisi pokok. Pada akhir eksperimen, kadar insiden penyakit ditunjukkan melalui simptom luaran dengan menunjukkan kesemua rawatan yang melibatkan Fusarium telah dijangkiti walaupun dirawat dengan *B. sphaericus* UPMB10. Secara signifikannya, serangan hanya bermula lewat di akhir eksperiment dan ini menunjukkan keupayaan *B. sphaericus* UPMB10 untuk melambatkan serangan penyakit tersebut. Inokulum pepejal *B. sphaericus* UPMB10 telah menunjukkan perlambatan kemunculan simptom, peratus insiden penyakit dan kadar epidemik yang rendah. Aplikasi kepada tanah telah menunjukkan *B. sphaericus* UPMB10 dengan cara pepejal telah memberikan ketahanan terbaik kepada penyakit layu fusarium. Kombinasi samada menggunakan aplikasi substrat cecair atau pepejal telah dipilih untuk penilaian terhadap penyakit di eksperimen rumah kaca. Di dalam kajian ini, didapati bahawa

inokulum dengan formulasi 100 g pepejal per pokok adalah optimum untuk memberikan ketahanan terbaik pada tahap antagonis pada dos yang sama. Kesimpulan menunjukkan *B. sphaericus* UPMB10 berpotensi tinggi untuk digunakan sebagai penggalak pertumbuhan pokok dan agen kawalan biologi dalam pengurusan penyakit layu Fusarium pada tanaman pisang.

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I certify that an Examination Committee met on 25th September 2008 to conduct the final examination of Illani Zuraihah Ibrahim on her thesis entitled "Application of *Bacillus sphaericus* UPMB10 for Growth Enhancement of Banana (*Musa* spp.var. Berangan) and Its Effects On Fusarium Wilt" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Univeristi Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded Master of Science.

Members of the Thesis Examination Committee were as follows:

Zaharah Abdul Rahman, Ph.D.
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Jugah Kadir, Ph.D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Halimi Mohd Saud, Ph.D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Nik Masdek Nik Hassan, Ph.D.
Lecturer
Horticultural Research Centre
Malaysian Agricultural Research and Development Institute
(External Examiner)

BUJANG KIM HUAT, PhD
Professor and Deputy Dean,
School of Graduate Studies,
Universiti Putra Malaysia.

Date: 19 February 2009

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Zulkifli Hj. Shamsuddin, Ph.D.
Professor,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Chairman)

Sariah Meon, Ph.D.
Professor,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Member)

Zakaria Wahab, Ph.D.
Associate Professor,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Member)

HASANAH MOHD GHAZALI, Ph.D.
Professor and Dean,
School of Graduate Studies,
Universiti Putra Malaysia.

Date: 9 April 2009

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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.

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Candidate: ILLANI ZURAIHAH IBRAHIM

Date:

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AUDPC	Area under Disease Progress Curve
cfu	Colony forming units
DI	Disease incidence
DS	Disease severity
DSI	Disease severity index
FOCR4	*Fusarium oxysporum* f.sp. *cubense* race 4
FSM	Fusarium selective media
FW	Fresh weight
GA	Gibberelic acid
IAA	Indole acetic acid
M	monit
PDB	Potato Dextrose Broth
PGPR	Plant growth promoting rhizobacteria
PIRG	Percentage of inhibition of radial growth
PSB	Phosphate solubilizing bacteria
RCBD	Randomised Complete Block Design
r_m	Epidemic rate
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth

CHAPTER 1

INTRODUCTION

Non-symbiotic rhizobacteria, associated with plant roots and able to enhance plant growth, known as plant growth promoting rhizobacteria (PGPR) has generated much interest in a wide range of research. It has impacts in different fields of study because it provides enormous information on different aspects of plant growth promotion and development while at the same time promises possible biological control solutions to pytopathogens of some plant diseases. At present, phytopathogens have caused a great loss in crop productivity and had to depend largely on chemicals, fumigation, steam-treatment and solarization of soils in order to control it (Gamliel and Katan, 1992). Earlier, it was believed that the advantages of chemical controls of diseases could be obtained without any deleterious environmental effects. Currently, researchers have realized that these chemical agents are both hazardous to animals and humans because they can persist and accumulate in natural ecosystems. Consequently, there is a strong desire to replace chemicals with biological approaches that are potentially more “friendly” to the environment in the long term. These biological approaches include the use of transgenic plants, which are resistant to one or more plant pathogens (Greenberg and Glick, 1993), or either to use of biocontrol plant growth-

promoting bacteria which can suppress or prevent the plant damage (O'Sullivan and O'Gara, 1992; Sivan and Chet 1992; Sutton and Pegg, 1993; Cook, 1993). Biocontrol seems to be a reliable alternative compared to chemical fungicides, which have raised serious concerns of food contamination and environmental pollution. Some saprotrophic bacteria can serve as excellent biocontrol agents against plant pathogens (Fernando *et al.,* 2005).

The introduction of beneficial microbes as biological control of soil borne plant pathogen as well as enhancement of plant growth has a considerable potential in agriculture. Biological control using antifungal rhizobacteria to suppress plant diseases offers a powerful alternative to the use of synthetic chemicals. Numerous studies have demonstrated the ability of several bacteria to suppress diseases caused by soil-borne and seed-borne plant pathogens (Weller, 1988; O'Sullivan and O'Gara, 1992; Whipps, 1997). The anti-fungal abilities of these beneficial microbes have been known since a few decades ago and there have been extensive efforts to use them for plant disease control and more recently on a commercialized scale.

The uniqueness of microorganisms has never been understood until their beneficial functions were discovered. It is a paradox that bacteria can be both beneficial and antagonistic towards a plant pathogen to maintain its life

cycle. In this manner, it is possible to introduce the beneficial microorganism as a biological control for soil borne plant pathogen as well as enhancement of plant growth. This creates a great implication on plant growth development through direct or indirect mechanisms (Glick *et al.,* 1999). A direct promotion of plant growth by PGPR is generally based on the ability of the bacteria to synthesize and facilitate nutrient uptake from the environment. Meanwhile indirect mechanism is through its effect on the phytopathogenic organism (usually fungus) by one or several mechanisms such as production of antibiotic and antifungal metabolite or enzyme that could lyse the fungal cell wall. It was believed that this advantage would lead to the potential of PGPR as a biocontrol agent besides stimulating plant growth. A local rhizobacterial strain, isolated from oil palm roots and identified as *Bacillus sphaericus* UPMB10 (*B. sphaericus* UPMB10), has shown great potential in promoting plant growth development and has been characterized for its optimum growth requirement, inoculum carrier (ground oil palm frond) and inoculum production technique (Premalalatha, 2006). *Bacillus sphaericus* is categorized as a free-living soil bacterium that can function as a plant growth promoting rhizobacteria (Kloepper *et al.,* 1989). A few studies have demonstrated that inoculation with *B. sphaericus* UPMB10 enhanced plant growth and fixed nitrogen with oil palm (Amir *et al.,* 2001 and banana (Mia *et al.,* 2000). The current research interest is to assess the ability of *B. sphaericus* UPMB10 as