UNIVERSITI PUTRA MALAYSIA

POTENTIAL OF *Bacillus subtilis* AS BIOLOGICAL CONTROL AGENT FOR RICE BLAST DISEASE IN MR219 RICE CULTIVAR

NARGES SOLEIMANI

FP 2014 5

POTENTIAL OF *Bacillus subtilis* AS BIOLOGICAL CONTROL AGENT FOR RICE BLAST DISEASE IN MR219 RICE CULTIVAR

By

NARGES SOLEIMANI

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

June 2014

COPYRIGHT

DEDICATION

This Thesis is dedicated to

The most precious people in my life; my mom and dad

Esmat and Naser

For their unconditional everlasting love

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

POTENTIAL OF *Bacillus subtilis* AS BIOLOGICAL CONTROL AGENT FOR RICE BLAST DISEASE IN MR219 RICE CULTIVAR

By

NARGES SOLEIMANI

June 2014

Chairman: Associate Professor Jugah B Kadir, PhD
Faculty: Agriculture

The present study focuses on the effect of *Bacillus subtilis* in rice blast disease reduction. This study was conducted in 3 experiments to investigate *B. subtilis* ability as a biological control agent in MR219 cultivar. Based on morphological characterization**,** *Bacillus* sp. were selected and purified on nutrient agar. Purified isolates were tested *in vitro* for antagonistic characteristics by dual culture assay. Out of all 54 bacterial isolates screened, 20 isolates showed some levels of antagonistic activity with two isolates having the strongest inhibitory activity of 57.40% and 62.96% based on percentage inhibition of radial growth (PIRG). Both strains were Gram-positive, rod shaped cells, motile, oxidase, catalase, urease, citrate and mannitol positive. PCR amplification using universal primers amplified a fragment of the expected size (900 bp) from the 16S rRNA gene. PCR products were purified and sequenced to identify the antagonistic strains. Strain B1 and B2 were identified with 98% similarity as *B. subtilis* strain QB928 (NC028520.1). Potential strains were subjected for culture filtrate test to detect the non-volatile diffusible inhibitors either as antibiotics, enzymes or other forms where culture filtrate of strain B1 and B2 were inoculated in nutrient broth and incubated for 7 days. The culture was centrifuged and filtered supernatant was incorporated into potato dextrose agar. The PIRG results suggested that administration of B1 and B2 strains can effectively inhibit mycelia growth by 75.43% and 64.79% respectively. Production of volatile compounds was determined using inverted culture plate method where mycelia growth of *Pyriculraia oryzae* was measured in incubated control, B1, and B2 treated petri dishes after 7 days. Results showed considerable reduction of antifungal activity for both strains of B1 and B2 which were 65.9% and 57.4% respectively, indicating volatile compound production in both strains culture. Glasshouse investigation showed the effect of both strains on

MR219 rice variety where *B. subtilis* application significantly (α=0.05) reduced the severity of disease, with the highest reduction of 57% which was recorded in treatments receiving strain B1. The area under the curve for severity of blast disease was assessed and the results found to be significantly different (*P< 0.05*) where 368.9 square units was recorded for strain B2 and 299.1 square units for strain B1. Disease progress rate in rice plants treated with strain B2 was higher (0.15 unit/day) than in strain B1 (0.12 unit/day) meaning that disease development was more slowly in strain B1. In plants which have received antagonistic bacteria the reduced severity of disease resulted in a significantly higher rate of photosynthesis (32.01 µmolm-2s-1) compared to other plants. Shoot dry weight of rice plants increased significantly (0.18 g) with *B. subtilis* application. Although both strains were effective in decreasing the intensity of blast, greater effectiveness was achieved through B1 application. Accordingly, on the basis of the results of this study, both candidates might be very promising biological control agents for blast control on MR219 cultivar where blast resistant cultivars have become susceptible.

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

POTENSI *Bacillus subtilis* SEBAGAI AGEN KAWALAN BIOLOGI UNTUK PENYAKIT KARAH PADI PADA KULTIVAR PADI MR219

Oleh

NARGES SOLEIMANI

Jun 2014

Pengerusi: Profesor Madya Jugah B Kadir, PhD
Fakulti : Pertanian

Kajian ini memberi tumpuan kepada kesan *Bacillus subtilis* dalam pengurangan penyakit karah padi. Tiga eksperimen telah dijalankan untuk mengkaji keupayaan *B. subtilis* sebagai agen kawalan biologi bagi kultivar MR219. Berdasarkan kepada pencirian morfologi, *Bacillus* sp. telah dipilih dan ditulenkan dengan menggunakan agar nutrien. Pencilan tulen ini telah diuji secara '*in vitro*' untuk pencirian antagonistik melalui cerakinan 'dual culture'. Daripada semua pencilan bakteria yang diperiksa, dua puluh pencilan menunjukkan beberapa tahap aktiviti antagonistik dengan dua pencilan mempunyai aktiviti perencatan yang kuat iaitu 57.40% dan 62.96%; berdasarkan kepada peratusan perencatan (PIRG). Kedua-dua strain adalah Gram-positif, mempunyai sel berbentuk rod, motil, oksidase, katalase, urease, sitrat dan positif kepada manitol. Amplifikasi PCR menggunakan primer universal mengaplikasi satu pecahan dari saiz jangkaan (900 bp); daripada gen 16S rRNA. Produk PCR telah ditulenkan dan disusun untuk mengenal pasti strain yang antagonistik. Strain B1 dan B2 telah dikenal pasti sebagai strain *B. subtilis* QB928 (NC028520.1) dengan 98% persamaan. Strain yang berpotensi diteruskan dengan ujian 'turasan kultur' untuk mengesan perencat resap yang tidak meruap sama ada sebagai antibiotik, enzim atau lain-lain bentuk di mana turasan kultur B1 dan B2 telah diinokulasikan di dalam brot nutrien dan dikekalkan selama 7 hari. Kultur ini telah diemparkan dan supernatan yang ditapis telah dimasukkan ke dalam 'Potato dextrose agar'. Keputusan PIRG menunjukkan strain B1 dan B2 mampu menghalang pertumbuhan mycelia secara berkesan dengan peratusan 75.43% dan 64.79%. Penghasilan sebatian meruap telah ditentukan dengan menggunakan kaedah plat kultur terbalik di mana

pertumbuhan miselium *Pyricularia oryzae* diukur pada inkubasi kawalan, B1, dan piring petri terawat B2; selepas 7 hari. Keputusan menunjukkan aktiviti antikulat berkurang bagi kedua-dua strain B1 dan B2 iaitu 65.9% dan 57.4%, membuktikan sebatian yang meruap telah dihasilkan oleh kedua-dua strain. Kajian rumah kaca menunjukkan kesan kedua-dua strain ke atas variati padi MR219 di mana aplikasi *B. subtilis* ($\alpha = 0.05$) dapat mengurangkan keterukan penyakit dengan ketara, dengan penurunan tertinggi yang direkodkan oleh rawatan B1, iaitu sebanyak 57%. Kawasan di bawah lengkung untuk keterukan penyakit karah padi telah dinilai dan keputusan menunjukkan perbezaan yang ketara ($P < 0.05$) di mana 368.9 unit^2 dicatatkan bagi B2 dan 299.1 unit^2 untuk B1. Kadar kemajuan penyakit pada padi yang dirawat dengan strain B2 adalah lebih tinggi (0.15 unit sehari) berbanding strain B1 (0.12 unit sehari) menunjukkan bahawa perkembangan penyakit adalah lebih perlahan dalam strain B1. Pokok yang dirawat dengan bakteria antagonistic menunjukkan pengurangan keterukan penyakit dan meningkatkan kadar fotosintesis (32.01 μmolm-2s-1) jika dibandingkan dengan pokok lain. Padi yang dirawat dengan *B. subtilis* menunjukkan peningkatan berat kering pucuk yang ketara (0.18 g). Walaupun kedua-dua strain mampu mengurangkan aktiviti karah padi, namun B1 telah menunjukkan keberkesanan yang sangat memberangsangkan. Berdasarkan hasil kajian ini, dapat disimpulkan bahawa kedua-dua strain sangat berguna untuk diapplikasikan sebagai agen kawalan biologi untuk mengawal penyakit karah pada kultivar MR219, di mana kultivar yang rintang kepada penyakit karah menjadi rentan.

ACKNOWLEDGEMENTS

I would like to express my heartfelt and deep appreciation to the chairman of my supervisory committee Associate. Prof. Dr.Jugah Kadir for his bounteous advices and suggestions and patience during this study. My profound gratitude is also extended to members of the supervisory committee, Prof. Dr. Abdul Shukor Juraimi for his suggestions and assistance. The assistance provided by MARDI research station in Seberang Perai Penang and RICE & INDUSTRIAL CROP RESEARCH CENTRE MARDI Serdang specialy Dr. Marzukhi Hashim is highly appreciated. Special thanks to all staffs of Pathology Lab and to all dear friends and lab mates especially Mahboubeh and Dr. Ali for their kindness and beautiful bond of helpfulness. I also would like to express my sincere thanks to R. Jafari a wonderful friend who for always being with me whenever help is needed. Last but not least I would like to offer my heartfelt thanks to my dearest parents and my lovely strong sister Mina who always believed in me and made me believe in myself to perform to my maximum ability.

I certify that a Thesis Examination Committee has met on 12 June 2014 to conduct the final examination of Narges Soleimani on her thesis entitled "Potential of *Bacillus subtilis* as Biological Control Agent for Rice Blast Disease in MR219 Rice Cultivar" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Nur Azura binti Adam, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Ganesan a/l Vadamalai, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Nur Ain Izzati binti Mohd Zainudin, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Chong Khim Phin, PhD
Senior Lecturer
Universiti Malaysia Sabah
Malaysia
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 21 July 2014

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

Jugah Bin Kadir, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Abdul Shukor Juraimi, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

_____

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

Date:

DECLARATION

Declaration by the student

I hereby confirm that:

- this thesis is my original work
- quotations, illustrations and citations have been duly referenced
- the thesis has not been submitted previously or comcurrently for any other degree at any institutions
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be owned from supervisor and deputy vice –chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date:_____

Name and Matric No.: Narges Soleimani GS28769

Declaration by Members of Supervisory committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature		Signature	
Name of Chairman of Supervisory Committee:	Associate Professor Dr. Jugah Bin Kadir	Name of Member of Supervisory Committee:	Professor Dr. Abdul Shukor Juraimi

TABLE OF CONTENTS