



**UNIVERSITI PUTRA MALAYSIA**

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

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**FP 2014 5**



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**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**

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## **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 *Pyricularia 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 ( $\alpha=0.05$ ) 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 ( $32.01 \mu\text{molm}^{-2}\text{s}^{-1}$ ) 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 sebagai 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 dituliskan 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 dituliskan 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 variasi 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<sup>2</sup> dicatatkan bagi B2 dan 299.1 unit<sup>2</sup> 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  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) 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 diaplikasikan sebagai agen kawalan biologi untuk mengawal penyakit karah pada kultivar MR219, di mana kultivar yang rintang kepada penyakit karah menjadi rentan.



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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.

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## **DECLARATION**

### **Declaration by the student**

I hereby confirm that:

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