

LACTIC ACID BACTERIA AS BIOCONTROL AGENT AGAINST
PATHOGENIC *FUSARIUM* SPECIES ON CHILLI PLANTS

AKARACHUSAN

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LACTIC ACID BACTERIA AS BIOCONTROL AGENT AGAINST
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This thesis submitted in fulfillment for the degree of

DOCTOR OF PHILOSOPHY

MICROBIAL BIOTECHNOLOGY IN AGRICULTURE

Faculty of Science and Technology

UNIVERSITI SAINS ISLAM MALAYSIA

MARCH 2016

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ACKNOWLEDGMENTS

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

In the Name of Allah, the Most Gracious, and the Most Merciful. All praises and thanks due to The Almighty Allah, Who is the intact source of knowledge and wisdom to me and all mankind. I owe my deep respect to Prophet Muhammad (Peace Be Upon Him), the city of knowledge and mercy for the entire universe. Almighty Allah has vastly blessed me through the people who have contributed to the completion of this thesis.

I wish to extend my greatest appreciation to my professor and thesis main supervisor, Dr. Zaiton Hassan, Associate Professor in the Faculty of Science and Technology, University Sains Islam Malaysia (USIM) for her suggestions, guidance, support, and encouragement in the completion of this thesis. Special thanks to Dr. Mohd. Nizam Lani, Senior Lecturer in the School of Food Science and Technology, University Malaysia Terengganu, Malaysia.

I wish to extend my deepest appreciation to my late parents Mr. Sattar Husain, Mrs. Fatibun Nisa and her sisters Khaliqun Nisa, Late Kaleemun Nisa and Late grandmother Mrs. Khadija w/o Deen Mohammad whose give me a lots of appreciation during my childhood life.

I tender my greatest thanks to my kin brothers, Mr. Afzal Husain and his wife Ashiya Khatoon and her sister Shakila Khatoon, Mr. Akmal Husain and his wife Mahiru Nisa and all my sisters for their energetic financial support, moral co-operation, and sincere help and patience. Without their helps, it has not been possible for me to make my head way in my whole study.

With pleasure and help deep sense of indebtedness, I acknowledge the invaluable guidance extended and financial support to me during research work with Zamalah scholarship for four semesters by University Science Islam Malaysia (USIM). It was the greatest support I have during my PHD study.

I wish to thank all my relatives, Late my uncle Sheikh Iqbal Saheb, Mr. Wasiullah Khan, Mr. Takseem Khan, Mr. Izhar Khan, Mr. Mazhar Khan, Mr. Ithar Khan, Mr. Mohammaddin Khan, Mr. Abdul Mannan Faizi, Mr. Abdul Hanan, Mr. Sameed Khan Mr. Hesamuddin, Mr. Abdul Basit, Mr. Mustaqeem Khan and late Mr. Lakmuddin Bhai for their moral support and sincere guidance in my study.

I also tender my deepest thanks to Maulana Abdul Aziz Slafi, Maulana Abdul Wajid Faizi, Mr. Iqbal Khan, Mr. Abdul Hafiz Khan, Sheikh Nasim sahib, Sheikh Abdul Wase Saheb, Maulana Mustaqeem Saheb, Sheikh Abdul Wahab Jamai Saheb, Sheikh Abdullah Faizi, Dr. Ehsan Saheb, Sheikh Qari Saukat Saheb Mohamadi, Sheikh Qumruddin Saheb, Mr. Sultan Bhatt, Mr. Farooque Bhatt, Wakeel Ahmad, Mr. Faiyaz Ahmad and Mr. Firoz Bhatt for their moral support and sincere guidance in my study.

ABSTRAK

Pokok cili mudah dijangkiti oleh *Fusarium* spp. dan mengakibatkan kerugian besar kepada tanaman cili di seluruh dunia. Oleh itu, kaedah yang sesuai diperlukan untuk mencegah dan mengurangkan jangkitan *Fusarium* spp. menjangkiti pokok cili. Kajian ini mengkaji bakteria asid laktik (LAB) sebagai bio-kawalan terhadap *Fusarium* species pada tanaman cili. Sebanyak 21 LAB telah dipencilkan daripada sumber-sumber yang berbeza iaitu 14 dari tanah, 7 daripada penapaian cili dan 3 dari kultur ATCC. Empat *Fusarium* spp. telah dipencilkan daripada sumber berbez dan telah dikenalpasti sebagai *Fusarium oxysporum* f. sp. *lycopersici*, *F. solani*, *F. acuminatum* dan *F. proliferatum*. Saringan menggunakan analisis dua lapisan menunjukkan 15 dari 21 pencilan LAB merencat *Fusarium* spp. dengan zon perencatan 4.0-60.0 mm selepas 48 jam eraman pada suhu 28°C. Supernatan *L. acidophilus* ATCC314, *L. plantarum* ATCC8014 dan tiga pencilan LAB lain yang dikenalpasti sebagai *Lactobacillus plantarum*1-LAB MSS1 (daripada tanah), *Pediococcus pentosaceus* 1-LAB MSS5 (daripada tanah) dan *Lactobacillus plantarum*1-LAB FF11 (daripada penapaian cili) menggunakan kit API dan 16rDNA penentuan genotip menunjukkan aktiviti antikulat yang kuat terhadap semua sasaran *Fusarium* spesies. Supernatan dari lima pencilan LAB menunjukkan perencatan pertumbuhan micelium terhadap semua *Fusarium* spp: terutama *F. solani* CS (daripada biji cili). Aktiviti antikulat daripada supernatan LAB dipengaruhi oleh rawatan enzim; aktiviti antikulat adalah dalam lingkungan 7.44-86.83% bergantung kepada LAB dan *Fusarium* spp. Pepsin mengurangkan aktiviti antikulat supernatant LAB-CFS MSS1, IDLAB6 dan FF11 terhadap *F. oxysporum* f. sp. *lycopersici*- CL. Aktiviti antikulat adalah sangat ketara berkurang ($P < 0.05$) dengan perubahan pH supernatan. Kehilangan aktiviti antikulat supernatan LAB telah diperhatikan pada pH 6 hingga 9, berkurang pada pH 2 dan 5, tetapi tetap pada pH 3 dan 4. Pemanasan LAB-CFS pada suhu 80°C dan 90°C selama 30 min dan 121°C selama 15 min menyebabkan kehilangan aktiviti antikulat dalam supernatant MSS5, IDLAB6 dan LAB-FF11 terhadap *F. acuminatum*-FC, manakala LAB yang lain masih menunjukkan aktiviti antikulat (3.09-97.75%) selepas 72 jam eraman pada 30°C. Aplikasi LAB-CFS kepada biji benih sebelum penyemaian mempertingkatkan percambahan kepada 97% tetapi tidak untuk benih yang dijangkiti kulat; di mana kadar percambahan menurun kepada 50%. Walaubagaimanapun, benih cili yang dirawat dengan supernatan LAB-FF11 dan sel-sel LAB-MSS1 menunjukkan kadar peningkatan percambahan yang ketara ($P < 0.05$) kepada kira-kira 98% walaupun benih ditanam ke tanah yang dijangkiti kulat. Pemanjangan pucuk dan akar dengan ketara ($P \leq 0.05$) diperhatikan apabila biji cili dirawat dengan CFS daripada LAB-FF11 sebelum penyemaian dengan purata 11.60 ± 0.57 cm selepas 16 hari eraman dalam keadaan gelap. Pokok-pokok dirawat dengan sel LAB MSS1 (kumpulan II) menunjukkan lebar kanopi 80.83 ± 10.51 cm, yang ketara ($P < 0.05$) lebih tinggi berbanding lebar canopy pokok cili yang dirawat dengan sel LAB FF11 (kumpulan IV) dan kumpulan lain. Kehadiran *F. solani* CS di dalam tanah menyebabkan pertumbuhan tidak normal pada pokok cili (Group VI) dan ketinggian tumbuhan mencapai 143.67 ± 0.41 cm daripada pokok-pokok yang dirawat dengan sel-sel LAB dan kumpulan pokok chili terkawal. Pokok-pokok yang dijangkiti *F. solani* CS menunjukkan peningkatan berat kering tumbuhan (77.32 g) dan kandungan air (77.03%) tetapi kurang daripada tumbuh-tumbuhan daripada kumpulan lain selepas 65 hari. Produktiviti (bilangan buah-buahan/pokok) daripada pokok-pokok yang dirawat

dengan LAB FF11 (kumpulan IV) adalah ketara ($P < 0.05$) lebih tinggi ($56.33 \pm 06.11\text{cm}$) berbanding dengan kumpulan lain. Pertumbuhan pokok-pokok tanpa LAB atau kulat adalah lambat dan produktiviti adalah 2%, masing masing. Pokok-pokok yang menerima rawatan LAB MSS1 (Kumpulan II), LAB MSS1 dan *F. solani* (kumpulan III), LAB FF11 (kumpulan IV), dan LAB FF11 dan *F. solani* (kumpulan VI) menghasilkan buah cili yang masak dan bertukar kepada merah dalam masa 90 hari, dan pertumbuhan pokok berterusan sehingga lebih dari 110 hari. Walaubagaimanapun, pokok yang dijangkiti *F. solani* (Group VI) mula menunjukkan kerosakan pokok selepas 52 hari. Kedua-dua *Fusarium* species. dan *L. plantarum* (MSS1 dan FF11) didapati bersifat endofitik dalam alam semula jadi. Rawatan benih dan tanah dengan LAB terpilih sama ada daripada sel atau supernatan menyebabkan pertumbuhan pesat pokok chili walaupun dengan kehadiran *Fusarium* spp. Oleh itu, kajian ini menunjukkan bahawa LAB terpilih *L. plantarum* (MSS1 dan FF11) sama ada sel atau supernatan boleh digunakan sebagai bio-kawalan terhadap pokok cili yang dijangkiti *Fusarium* species. Perawatan benih atau tanah menggunakan LAB sama ada dalam bentuk sel atau supernatan dapat mempertingkatkan pertumbuhan pokok, meningkatkan produktiviti pokok cili dan juga menghalang pertumbuhan *F. solani*.

ABSTRACT

Chilli plants are easily infected by *Fusarium* species and cause enormous loss of food products as well as plants worldwide. Therefore, a suitable method is required for prevention and reduction of *Fusarium* species affecting chilli plants. This research explored the possibility of using lactic acid bacteria (LAB) as bio-control against *Fusarium* species on chilli plants. A total of 21 LABs were isolated from different sources 14 from soil, 7 from fermented chilli fruits and 3 ATCC strains. Four *Fusarium* species were isolated from different plants parts and identified as *Fusarium oxysporum* f. sp. *lycopersici*, *F. solani*, *F. acuminatum* and *F. proliferatum*. Screening using dual overlay assay showed that 14 from 21 LAB isolates inhibited *Fusarium* species with zone of inhibition 4.0 to 60.0 mm after 48 h incubation at 28°C. The supernatants of *Lb. acidophilus* ATCC314, *Lb. plantarum* ATCC8014 and three other LAB isolates were identified as *Lb. plantarum*1-LAB MSS1 (isolated from soil), *Pediococcus pentosaceus*1-LAB MSS5 (isolated from soil) and *Lb. plantarum*1-LAB FF11 (isolated from fermented chilli fruits) using API Kit and 16rDNA genotypic identification showed strong antifungal activity against all targeted *Fusarium* species. The supernatants of five LAB isolates showed mycelial growth inhibition against all *Fusarium* species especially *F. solani* CS (isolated from chilli seeds). The antifungal activity of the LAB supernatants was affected by enzyme treatment, the antifungal activity was in the range of 7.44 to 86.83% depending on LAB and *Fusarium* species. Pepsin reduced the antifungal activity of supernatants LAB-CFS MSS1, IDLAB6 and FF11 against *F. oxysporum* f. sp. *lycopersici*- CL. The antifungal activity was significantly ($P < 0.05$) reduced by pH of supernatant. Loss of antifungal activity of LAB supernatant was observed at pH 6 to 9, reduced at pH 2 and 5, but maintained at pH 3 and 4. Heating LAB-CFS at temperatures 80°C and 90°C for 30 min and 121°C for 15 min resulted in loss of antifungal activity in MSS5, IDLAB6 and FF11 against *F. acuminatum*-FC, while other LAB maintained the antifungal activity (3.09 to 97.75%) after 72 h incubation at 30°C. Application of LAB-CFS to seed prior to sowing enhanced seed germination by 97% but not for seeds infected with the fungi; the germination rate was reduced to 50.00%. However, chilli seeds treated with supernatant of LAB-FF11 and cells of LAB-MSS1 significantly ($P < 0.05$) increased the germination rate to about 98% even when the seeds were sowed in soils infected with the fungi. Significant shoot and root elongation was observed when the seeds were treated with CFS of LAB-FF11 before sowing with an average of 11.60 ± 0.57 cm after 16 day incubation in the dark. Plants treated with LAB MSS1 (group II) showed broadest width canopy of 80.83 ± 10.51 cm, significantly ($P < 0.05$) higher compared to LAB FF11 (group IV) and other groups. Inoculating *F. solani*-CS to soil resulted in abnormal growth of chilli plants (Group VI) and plant height reached 143.67 ± 0.41 cm than plants treated with LAB cells and control plants. Fungi infected plants showed an increase in dry weight of plants (77.32 g) and the water content was 77.03% which was less than plants from others group after 65 days of transplanting. The productivity (number of fruits/plant) was significantly ($P < 0.05$) higher (56.33 ± 06.11) in plants treated with LAB FF11 (group IV) compare to other groups. Growth of plants without LAB or fungi was slow and productivity was 2% per plant. Plants receiving treatments LAB MSS1 (groups II) LAB MSS1 and *F. solani* (group III), LAB FF11 (group IV), and LAB FF11 and *F. solani* (group VI) produced chilli fruits which ripened and turned to red within 90 d, and plant growth continued until more than 110 d. However,

plant infected with *F. solani* (Group VI) started to show plant death after 65 days. Both *Fusarium* species and the *Lb. plantarum* (MSS1 and FF11) LAB were found to be endophytic in nature. Treatment of seeds and soil with selected LAB either as cells or supernatant resulted in rapid growth of plants in the presence of *Fusarium* species. Therefore, this study demonstrated that selected LAB either cells or their supernatants could be used as bio-control against *Fusarium* species infecting chilli plants. Treating the seeds or soil using LAB either cells or supernatants enhanced plant growth, improved the productivity of chilli plants and also suppressed the growth of *F. solani*.

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المخلص

الفلفل نبات يصاب بسهولة عن طريق فطر الفيوزاريوم. وتسبب خسائر هائلة من المواد الغذائية وكذلك محطات في جميع أنحاء العالم. لذلك، لا بد من طريقة مناسبة للوقاية والحد من الفيوزاريوم النياية. التي تؤثر على نبات الفلفل. اكتشف هذا البحث إمكانية استخدام بكتيريا حمض اللبنيك (LAB) بكتيريا حامض اللبنيك للمكافحة الحيوية ضد الفيوزاريوم النياية. لنبات الفلفل. تم عزل بكتيريا حامض من 21 مصدر مختلف 14 من التربة، و 7 من ثمار الفلفل المخمرة و 3 سلالات أي تي سي سي. أربعة الفيوزاريوم النياية. تم عزل من أجزاء النباتات المختلفة والتعرف على الفيوزاريوم أوكسيسبورم و. س lycopersici ، F. solani، F. مؤنف وواو. *proliferatum*. يعزل تحول دون الفيوزاريوم النياية

وتم استخدام طريقه ديول او فرلي لقياس نسبة التثبيط الحيوي لبكتيريا حامض اللبنيك وكان نطاق التثبيط يتراوح ما بين اربعة الى ستة بالمئه ملم بعد 48 ساعه من التحضين بدرجه 28 مئوية. الراشح من بكتيريا حامض اللبنيك لكتوباسلس اسيدوفلس اي تي سي سي 314

لكتوباسلس بلانتيريوم اي تي سي سي 8013 وثلاث عزلات اخرى شخصت على اساس انها لكتوباسلس بلانتيريوم عزلت من التربة بيدوكوكس بينتو ساكيس عزلت من التربة لكتوباسلس بلانتيريوم عزلت من نبات الفلفل وتم استخدام اي بي اي كيت و 16 دي ان اينشيط مضاد قوي ضد انواع الفطر المختلفة راشح عزلات بكتيرية حامض اللبنيك اظهرت نمو مضاد لانواع الفطريات المعزولة من بذور الفلفل الفعالية المضادة للفطريات من راشح بكتيرية حامض اللبنيك وتراوحت الفعالية ما بين 7.44 الى 86.83

اعتمادا على بكتيرية حامض اللبنيك وانواع الفطر اختزل البسبين الفعالية الفطرية للانواع المختلفة من بكتيرية حامض اللبنيك وكان تعزيز نمو النبات، وتحسين إنتاجية نباتات الفلفل، انخفاض درجة الحموضة من طاف. وقد لوحظ فقدان النشاط مضاد للفطريات من لطافي بكتيريا حامض اللبنيك في درجة الحموضة 6-9.

التدفئة LAB-CFS في درجات حرارة 80°C و 90°C لمدة 30 دقيقة و 121°C لمدة 15 دقيقة أدى إلى فقدان النشاط مضاد للفطريات في MSS5 ، IDLAB6 و LAB-FF11 ضد واو مؤنف FC-، في حين حافظت LAB آخر نشاط مضاد للفطريات (3،09 حتي 97،75٪) بعد 72 ساعة حضانة في 30°C تطبيق LAB-CFS إلى البذور قبل البذر تعزيز إنبات البذور بنسبة 97٪ ولكن ليس لبذور مصابة الفطريات. تم تخفيض نسبة الإنبات إلى 50.00٪. ومع ذلك، بذور الفلفل تعامل مع طاف من LAB-FF11 وخلايا LAB-MSS1 بشكل ملحوظ (p≤0.5) زادت نسبة الإنبات إلى حوالي 98٪ حتى عندما تم زرع البذور في التربة المصابة مع الفطريات

وقد لوحظ زياده كبيرة واستطالة الجذر عندما تم علاج البذور مع لجنة الأمن الغذائي من LAB-FF11 قبل البذر بمتوسط 11.60 ± 0.57 سم بعد الحضانة 16 يوم في الظلام. أظهرت النباتات المعاملة مع LAB MSS1 (المجموعة الثانية) أوسع مظلة عرض 80.83 ± 10.51 سم، إلى حد كبير (p≤0.5) أعلى مقارنة LAB (FF11 المجموعة الرابعة) وغيرها من الجماعات. بتلقيح واو سولاني CS-للتربة أدى إلى نمو غير طبيعي للنباتات الفلفل (المجموعة السادسة) وارتفاع النبات وصلت 143.67 ± 0.41 سم من النباتات تعامل مع الخلايا LAB ومحطات السيطرة. وأظهرت الفطريات النباتات المصابة زيادة في الوزن الجاف للنباتات (77.32 غرام) ومحتوى الماء كان 77.03٪ والذي كان أقل من النباتات من مجموعة أخرى بعد 65 يوما من زراعة.

وكانت الإنتاجية (عدد الثمار / نبات) بشكل ملحوظ (p≤0.5) أعلى (56.33 ± 06.11) في النباتات المعاملة مع LAB FF11 (المجموعة الرابعة) مقارنة المجموعات الأخرى. كان نمو النباتات دون LAB أو الفطريات بطيئة وكانت إنتاجية 2٪ لكل نبات. النباتات تلقي العلاج LAB MSS1 المجموعتين الثانية (LAB MSS1 و F.solani المجموعة الثالثة)، (LAB FF11 المجموعة الرابعة)، و (LAB FF11 F.solani المجموعة السادسة) إنتاج ثمار الفلفل التي نضجت وتحولت إلى اللون الأحمر في غضون 90 أيام، ونمو النبات

وستمرت حتى أكثر من 110 يوما. ومع ذلك، مصنع المصابين واو سولاني (المجموعة السادسة) بدأت تظهر موت النبات بعد 52 يوما. كلا الفيوزاريوم النياية. وعثر على MSS1 و *plantarum* Lb. (MSS1) LAB و FF11 أن

نابوت داخلي في الطبيعة. معالجة البذور والتربة مع LAB اختيار إما خلايا أو طاف أدت إلى النمو السريع للنباتات في وجود فطر *Fusarium* النيابة. ولذلك، أظهرت هذه الدراسة أن LAB اختيار إما الخلايا و supernatants يمكن أن تستخدم لمكافحة البيولوجية ضد الفيوزاريوم النيابة. إصابة النباتات الفلفل. معالجة البذور أو التربة باستخدام LAB إما خلايا أو supernatants تعزيز نمو النبات، وتحسين إنتاجية النباتات الفلفل، وقمعت أيضا نمو واو سولاني

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LIST OF ABBREVIATIONS

µg	Micro-grams
µm	Micro-metre
CaCO ₃	Calcium carbonate
CFS	Cell free supernatants
CFS-LAB	Cell free supernatants of lactic acid bacteria
CFU	Colony forming unit
cm	Centimeter
DNA	deoxyribonucleic acid
Fungi-CL	Fungi (<i>F. oxysporum</i> f. sp. <i>lycopersici</i>) isolated from Chilli leaf (CL)
Fungi-CS	Fungi (<i>F. solani</i>) isolated from Chilli seed (CS)
Fungi-FC	Fruit of chilli
Fungi-LR	Leaf of rose
g	Grams
g*	Growth
GI	Growth inhibition
GRAS	Generally recognized as safe
GS	Germination % of seeds
h	Hours
H ₂ O ₂	Hydrogen peroxide
IDLAB6	Identified Lactic acid bacteria (<i>Lb. acidophilus</i> ATCC8014)
IDLAB7	Identified Lactic acid bacteria (<i>Lb. plantarum</i> ATCC314)
LAB	Lactic acid bacteria
LAB-CFS	Lactic acid bacteria cell free supernatant
LAB-FCF	Lactic acid bacteria (<i>Lb. plantarum</i> 1) isolated from fermented chilli fruit
LABMSS	Lactic acid bacteria Malaysian soil sample
MEB	Malt Extract Broth
mL	Milliliter
mm	Milimetre
MRSA	De man Rogosa Shape Agar
MRSB	De man Rogosa Shape Broth
nm	Nanometer
OD	Optical Density
sp.	specie
TC	Total Control

TT	Total Treatment
PDA	Potato Dextrose Agar
PDB	Potato dextrose broth
PGPR	Plant growth promoting rhizobacteria
rDNA	Recombinant deoxyribonucleic acid
SMA	Skim Milk Agar
TC	Total control
TNGS	Total number of germinated seeds
TNTS	Total number of treated seeds
TT	Total treatment

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