

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการการวิจัยแห่งชาติ



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BIOLOGICAL CONTROL OF BACTERIAL WILT IN  
PATHUMMA (*Curcuma allismatifolia* Cagnep.)

SARAN PROMSAI

DOCTOR OF PHILOSOPHY  
IN APPLIED MICROBIOLOGY

THE GRADUATE SCHOOL  
CHIANG MAI UNIVERSITY  
SEPTEMBER 2011

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**A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN  
PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF  
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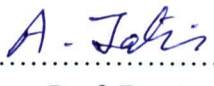
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FOR THE DEGREE OF DOCTOR OF PHILOSOPHY  
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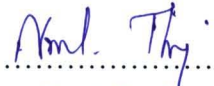
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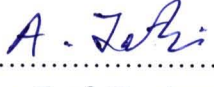
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## ABSTRACT

**E**<sub>47367</sub>

Forty-five isolates of wilt causing bacteria were isolated from infected Pathumma rhizomes using TZC medium. Ten bacterial isolates, namely PRZ, PT1B, PT1J, PT2X, D1, RRD, RT1S, Rh1-1 were identified as *Enterobacter* spp., Tu1-1 was identified as *Klebsiella* sp. and Tu1-2 was identified as *Pseudomonas* sp. by conventional and molecular methods. These bacteria were determined for their abilities to cause high disease severity in Pathumma plant both *in vivo* and laboratory bioassays. Fifteen bacterial isolates, namely PRZ, PT1B, PT1J, PT2X, RRD, RT1K, RT1S, RT2R, C4, D1, Rh1-1, Rh3-1, Tu1-1, Tu2-1 and R1512 were evaluated the persistence in natural soil without Pathumma plants. It was found that these bacteria had 42-70% survival rate after incubation for 1 year.

For the study of the adhesion of wilt causing bacteria in Pathumma tissue, *E. asburiae* PT1J was selected to investigate due to its high level of disease incidence. The infected pseudostems were observed changes under light compound and scanning electron microscope. The electron microscopic studies clearly showed the bacteria adhesion and structural changes of plant tissues. This bacterium could adhere to the vascular bundle walls and caused plant tissue shrunken.

One hundred and two bacterial isolates were isolated from soil samples collected from different sites in Thailand using TSA medium. After testing their ability to inhibit growth of the pathogenic bacteria using paper disc diffusion method, it was found that four isolates namely SP15, SP38, SP46 and SP58 had the highest ability to inhibit growth of wilt causing bacteria. From biochemical and molecular identification, the isolates SP15, SP38, SP46 and SP58 were *Bacillus subtilis*, *Pseudomonas mosselii*, *Pseudomonas mosselii* and *Pseudomonas aeruginosa*, respectively. The optimal conditions of inhibiting substances from the isolate SP15 was 30°C at pH 8 in modified TSB medium containing 0.5% (w/v) glucose and 2% (w/v) peptone, SP38 was 25°C at pH 7 in modified TSB medium containing 0.5% (w/v) sucrose and 2% (w/v) peptone, SP46 was 25°C at pH 7 in modified TSB medium containing 0.5% (w/v) glucose and 1.5% (w/v) peptone, and SP58 was 25°C at pH 7 in modified TSB medium containing 0.5% (w/v) sucrose and 1.5% (w/v) peptone.

Characterization of inhibiting substances produced by antagonistic bacteria was found that all antagonists could produce both hydroxamate-type and catecholate-type siderophore. Among four antagonists, only three strains *Ps. mosselii* SP38, *Ps.*



*mosselii* SP46 and *Ps. aeruginosa* SP58 showed the ability to produce phenazine derivatives.

The three experimental designs were used to evaluate the ability to control bacterial wilt in pots of pathumma. Experiment 1, both antagonistic and pathogenic bacteria were co-applied to pathumma rhizomes and to soil in pots before cultivation. Experiment 2, both antagonistic and pathogenic bacteria were co-applied to shooting pathumma. Experiment 3, pathogenic bacteria were applied to rhizomes and to soil before pathumma cultivation while the mixed culture of antagonistic bacteria were added to the plant pots after shooting. All experiments were conducted three times consecutively in 2008, 2009 and 2010. The results revealed that experiment 1 and 2 had the disease incidence of 0-33% while experiment 3 had the disease incidence of 33-67%. In all experiments, the bacterial cell numbers of antagonistic mixture were declined by 5-15% on average while pathogenic bacteria PT1J, PT2X, D2, RRD, RT1S and R227 were declined by 30, 35, 25, 40, 30 and 30% on average, respectively. However, all experiments could reduce wilt disease compared with the disease plant controls which were not treated with antagonistic bacteria.

Molasses and soil were found to be most suitable carrier materials for the optimal formulation for all antagonistic bacteria. The cell numbers of each antagonistic bacterial isolate were  $1 \times 10^4$ - $1 \times 10^5$  cfu/g in molasses or soils after 2 months of incubation.

**Keywords:** biological control, *Curcuma alismatifolia*, Pathumma, wilt disease, antagonistic bacteria, *Enterobacter* spp.



## ชื่อเรื่องวิทยานิพนธ์

การควบคุมโรคเหี่ยวในปทุมมา (*Curcuma alismatifolia* Gagnep.) ที่เกิดจากแบคทีเรียโดยวิธีทางชีวภาพ

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## บทคัดย่อ

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แบคทีเรียก่อโรคเหี่ยวในปทุมมาถูกคัดแยกได้จากหัวพันธุ์ที่เป็นโรคเหี่ยว โดยใช้อาหาร TZC พบว่าสามารถแยกเชื้อได้ทั้งสิ้น 45 ไอโซเลท ในจำนวนนี้มี 10 ไอโซเลทที่มีความสามารถในการก่อโรคได้รุนแรงที่สุด โดยการทดสอบการก่อโรคในแปลงปลูกปทุมมาและในห้องปฏิบัติการ ซึ่งได้แก่ *Enterobacter* sp. PRZ, PT1B, PT1J, PT2X, D1, RRD, RT1S และ Rh1-1, *Klebsiella* sp. Tu1-1 และ *Pseudomonas* sp. Tu1-2 เมื่อศึกษาการคงอยู่ในดินที่ปราศจากพืชอาศัยของแบคทีเรียก่อโรคเหี่ยวจำนวน 15 ไอโซเลท ได้แก่ PRZ, PT1B, PT1J, PT2X, RRD, RT1K, RT1S, RT2R, C4, D1, Rh1-1, Rh3-1, Tu1-1, Tu2-1 และ R1512 พบว่ามีอัตราการรอดชีวิต 42-70% เมื่อเวลาผ่านไป 1 ปี

ในการศึกษาการเกาะติดเนื้อเยื่อพืชของ *E. asburiae* PT1J ซึ่งสามารถก่อโรคได้รุนแรง โดยการสังเกตภายใต้กล้องจุลทรรศน์แบบส่องกราด พบว่าแบคทีเรียสามารถเกาะติดเนื้อเยื่อพืชได้ดี เนื้อเยื่อพืชมีการหดตัวเมื่อเปรียบเทียบกับกลุ่มควบคุมที่ไม่มีเชื้อ

ในการแยกแบคทีเรียจากดินบริเวณต่างๆ ของประเทศไทยด้วยอาหาร TSA พบว่าสามารถแยกได้ 102 ไอโซเลท แบคทีเรียทั้งหมดถูกนำไปทดสอบการยับยั้งการเจริญของแบคทีเรียก่อโรคเหี่ยวด้วยวิธี paper disc diffusion พบว่ามี 4 ไอโซเลท ได้แก่ *Bacillus subtilis* SP15, *Pseudomonas mosselii* SP38, *Pseudomonas mosselii* SP46 และ *Pseudomonas aeruginosa* SP58 มีความสามารถในการสร้างสารยับยั้งการเจริญของแบคทีเรียก่อโรคเหี่ยว โดยมีสภาวะที่เหมาะสมคือ *B. subtilis*

SP15 สร้างสารยับยั้งได้ดีที่อุณหภูมิ 30 องศาเซลเซียส ในอาหารเลี้ยงเชื้อ pH 8 ที่มีส่วนประกอบของ 0.5% (w/v) glucose และ 2% (w/v) peptone สำหรับ *Ps. mosselii* SP38 คือ ที่อุณหภูมิ 25 องศาเซลเซียส ในอาหารเลี้ยงเชื้อ pH 7 ที่มีส่วนประกอบของ 0.5% (w/v) sucrose และ 2% (w/v) peptone สำหรับ *Ps. mosselii* SP46 คือ ที่อุณหภูมิ 25 องศาเซลเซียส ในอาหารเลี้ยงเชื้อ pH 7 ที่มีส่วนประกอบของ 0.5% (w/v) glucose และ 1.5% (w/v) peptone และสำหรับ *Ps. aeruginosa* SP58 คือ ที่อุณหภูมิ 25 องศาเซลเซียส ในอาหารเลี้ยงเชื้อ pH 7 ที่มีส่วนประกอบของ 0.5% (w/v) sucrose และ 1.5% (w/v) peptone

ในการศึกษาสารยับยั้งที่ผลิตโดยแบคทีเรียปฏิปักษ์ SP15, SP38, SP46 และ SP58 พบว่าทั้ง 4 ไอโซเลทสามารถสร้างสาร siderophore ชนิด hydroxamate และ catecholate นอกจากนี้ยังพบว่าแบคทีเรียปฏิปักษ์ SP38, SP46 และ SP58 สามารถผลิตสารในกลุ่ม phenazine ได้

เมื่อนำแบคทีเรียปฏิปักษ์ไปทดสอบความสามารถในการควบคุมแบคทีเรียก่อโรคเหี่ยวในแปลงปลูก โดยแบ่งการทดลองเป็น 3 กลุ่มคือ กลุ่มที่ 1 ทำการเพาะเชื้อแบคทีเรียปฏิปักษ์พร้อมกับแบคทีเรียก่อโรคเหี่ยวตอนเริ่มปลูกปทุมมา กลุ่มที่ 2 ทำการเพาะเชื้อแบคทีเรียปฏิปักษ์พร้อมกับแบคทีเรียก่อโรคเหี่ยวตอนต้นปทุมมางอก และกลุ่มที่ 3 ทำการเพาะเชื้อแบคทีเรียก่อโรคเหี่ยวตอนเริ่มปลูกปทุมมา และตามด้วยแบคทีเรียปฏิปักษ์ภายหลังจากต้นปทุมมางอก ทำการทดลองทั้งหมด 3 ครั้งในปี 2008, 2009 และ 2010 ในการวิจัยพบว่า กลุ่มที่ 1 และ 2 มีอัตราการเกิดโรค 0-33% ในขณะที่กลุ่มที่ 3 มีอัตราการเกิดโรค 33-67% ในการตรวจสอบหาปริมาณเชื้อแบคทีเรียปฏิปักษ์และแบคทีเรียก่อโรคเหี่ยวในทั้งสามกลุ่มการทดลอง พบว่าปริมาณเชื้อผสมของแบคทีเรียปฏิปักษ์ลดลง 5-15% ส่วนแบคทีเรียก่อโรคเหี่ยว PT1J, PT2X, D1, RRD, RT1S และ R227 ลดลง 30, 35, 25, 40, 30 และ 30% ตามลำดับ อย่างไรก็ตามทั้ง 3 กลุ่มการทดลองสามารถลดการเกิดโรคเหี่ยวได้เมื่อเทียบกับกลุ่มควบคุมที่มีการเพาะเชื้อแบคทีเรียก่อโรคเหี่ยวเพียงอย่างเดียว

ในการศึกษาหาส่วนผสมที่เหมาะสมต่อการเพาะเลี้ยงแบคทีเรียปฏิปักษ์พบว่า กากน้ำตาลและดินมีความเหมาะสมที่สุด โดยพบปริมาณเชื้อแบคทีเรียปฏิปักษ์ทุกไอโซเลทอยู่ระหว่าง  $1 \times 10^4$  -  $1 \times 10^5$  cfu/g เมื่อเวลาผ่านไป 2 เดือน

**คำสำคัญ:** การควบคุมทางชีวภาพ, ปทุมมา, โรคเหี่ยว, แบคทีเรียปฏิปักษ์, *Enterobacter* spp.

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## ABBREVIATIONS AND SYMBOLS

%	=	percent
°C	=	degree Celsius
bp	=	base pair
cm	=	centrimeter
cfu/g	=	colony forming unit per gram
cfu/ml	=	colony forming unit per milliliter
cv	=	cultivar
g	=	gram
g/l	=	gram per liter
kg/ha	=	kilogram per hectare
l	=	liter
m	=	meter
mg	=	milligram
mm	=	millimeter
mM	=	millimolar
μl	=	microliter
μm	=	micrometer
μM	=	micromolar
M	=	molar
OD	=	optical density
pH	=	power of hydrogen ion



v/v = volume by volume

w/v = weight by volume