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ที่ต่อต้านเชื้อก่อโรคและต่อต้านการอักเสบ



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ISOLATION AND CHARACTERIZATION OF PROBIOTIC *LACTOBACILLUS* SPP.
WITH ANTI-PATHOGENIC AND ANTI-INFLAMMATORY ACTIVITIES

Mrs. Malai Taweechotipatr

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Medical Microbiology
(Interdisciplinary Program)

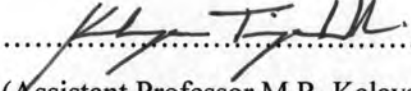
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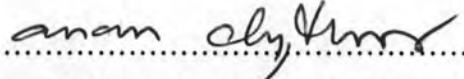
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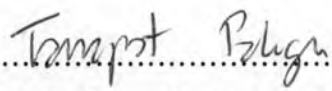
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แลคโตบาซิลลัส สปีชีส์ มีประโยชน์ในการนำมาใช้เป็นโพรไบโอติก ในการศึกษาครั้งนี้ ได้แยกเชื้อแลคโตบาซิลลัสจากอุจจาระของอาสาสมัครที่มีสุขภาพดี เพื่อนำมาศึกษาคุณสมบัติของโพรไบโอติก ด้านความสามารถในการยับยั้งการเจริญของเชื้อก่อโรคในระบบทางเดินอาหาร และความสามารถในด้านการควบคุมการสร้าง tumor necrosis factor- α (TNF- α) ตลอดจนการศึกษาคุณลักษณะของแลคโตบาซิลลัส สายพันธุ์ที่คัดเลือกได้

จากการนำแลคโตบาซิลลัสประมาณ 510 สายพันธุ์ มาทดสอบความสามารถในการยับยั้งการเจริญของเชื้อก่อโรคในระบบทางเดินอาหาร 10 สายพันธุ์ ผลการศึกษาโดยวิธี agar well diffusion assay พบว่าเชื้อแลคโตบาซิลลัส 4 สายพันธุ์ สามารถยับยั้ง เชื้อ *Vibrio cholerae* non O1 ได้เล็กน้อย และจากการทดสอบโดยวิธี agar spot method พบว่าเชื้อแลคโตบาซิลลัส 114 สายพันธุ์ สามารถยับยั้ง เชื้อ *Vibrio cholerae* ได้เล็กน้อย และในกลุ่มนี้มี 32 สายพันธุ์ สามารถยับยั้งเชื้อ *Salmonella enterica* ได้เล็กน้อย

จากการคัดเลือกแลคโตบาซิลลัสแบบสุ่มจำนวน 46 สายพันธุ์ เพื่อทดสอบความสามารถในการควบคุมการสร้าง TNF- α ในเซลล์โมโนไซต์ THP-1 ที่กระตุ้นด้วย lipopolysaccharide (LPS) พบว่าแลคโตบาซิลลัส 12 สายพันธุ์ สามารถยับยั้งการสร้าง TNF- α ได้ต่างกันอย่างมีนัยสำคัญ สายพันธุ์ TH58 สามารถยับยั้งการสร้าง TNF- α ได้สูงสุดประมาณ 70-80% อย่างไรก็ตามสายพันธุ์นี้ไม่มีผลต่อการควบคุมการกระตุ้น nuclear factor kappa B (NF- κ B)

การศึกษาคุณลักษณะของเชื้อโดยใช้ API 50 CHL การหาลำดับเบสของยีน 16S rRNA pyrosequencing และ การจัดกลุ่มด้วย rep-PCR พบว่า แลคโตบาซิลลัสทั้ง 4 สายพันธุ์ ที่ยับยั้ง *Vibrio cholerae* non O1 เป็น *Lactobacillus plantarum* เชื้อ 12 สายพันธุ์ที่ยับยั้งการสร้าง TNF- α พบว่าเป็น *Lactobacillus plantarum* 10 สายพันธุ์, *Lactobacillus salivarius* 1 สายพันธุ์ และสายพันธุ์ TH58 เป็น *Lactobacillus saerimneri* จากการศึกษาความสัมพันธ์ทางพันธุกรรมพบว่า *Lactobacillus saerimneri* มีความแตกต่างจาก *Lactobacillus plantarum* และ *Lactobacillus salivarius* ซึ่งโดยวิธี rep-PCR พบว่ามีความคล้ายคลึงกันเพียง 60-78% เป็นที่น่าสนใจว่าแลคโตบาซิลลัส (สายพันธุ์ TH58) ซึ่งยับยั้งการสร้าง TNF- α ได้สูงสุดนั้นเป็น *Lactobacillus saerimneri* ไม่เคยมีรายงานว่าแยกได้จากคนและสามารถยับยั้งการสร้าง TNF- α ได้

สาขาวิชาจุลชีววิทยาทางการแพทย์ (สหสาขาวิชา)

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ลายมือชื่อนิสิต..... *มาลัย ทวีโชคภัทร์*

ลายมือชื่ออาจารย์ที่ปรึกษา..... *ดร.สมหญิง ธีมวาสร*

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม..... *ดร.นพ. เจมส์ เวอร์ชาโลวิก*

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MALAI TAWEECHOTIPATR : ISOLATION AND CHARACTERIZATION OF PROBIOTIC *LACTOBACILLUS* SPP. WITH ANTI - PATHOGENIC AND ANTI-INFLAMMATORY ACTIVITIES. THESIS ADVISOR : ASSOC. PROF. SOMYING TUMWASORN, Ph.D., THESIS COADVISOR : ASSOC. PROF. JAMES VERSALOVIC, M.D., Ph.D., 210 pp.

Lactobacillus species have beneficial effects as probiotics. In this study, they were isolated from feces of healthy human volunteers to investigate for their two main probiotic properties: inhibition of gastrointestinal pathogens and modulation of tumor necrosis factor- α (TNF- α) productions as well as characterization of selected strains.

Five hundreds and ten *Lactobacillus* isolates were tested for antagonistic activity against ten gastrointestinal pathogens. The results demonstrated that 4 isolates displayed weak inhibitory activities against *Vibrio cholerae* non O1 by agar well diffusion assay. The weak inhibitory activities were observed in 114 of 437 isolates toward *Vibrio cholerae* and 32 of 114 isolates toward *Salmonella enterica* by agar spot method.

Forty-six isolates were randomly selected and investigated for the modulation of TNF- α production in THP-1 monocytic cells activated with lipopolysaccharide (LPS). The result revealed that 12 isolates significantly inhibited TNF- α production in varying degrees. Strain TH58 displayed the most potent TNF- α inhibitory activity by 70-80%. However, this strain had no effect on nuclear factor kappa B (NF- κ B) activation.

On the basis of phenotypic and genotypic characteristic including API 50 CHL, 16S rRNA gene sequencing, pyrosequencing and rep-PCR genotyping, 4 anti-pathogenic strains were identified as *Lactobacillus plantarum*. Out of 12 TNF- α inhibitory strains, 10 were identified as *Lactobacillus plantarum*, one as *Lactobacillus salivarius* and TH58 strain as *Lactobacillus saerimneri*. Phylogenetic relationships demonstrated *Lactobacillus saerimneri* distinguish from *Lactobacillus plantarum* and *Lactobacillus salivarius* which displayed 60-78% similarity by rep-PCR. It is interesting to note that *Lactobacillus* TH58 strain with the most potent TNF- α inhibitory activity was identified as *Lactobacillus saerimneri* which has never been reported as the isolate from human origin and exhibited TNF- α inhibitory activity.

Field of study Medical Microbiology
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LIST OF ABBREVIATIONS

CFU	=	colony forming unit
LMM	=	low-molecular-mass
HMM	=	high-molecular-mass
H ₂ O ₂	=	hydrogen peroxide
CO ₂	=	carbon dioxide
kDa	=	kilodalton
MIC	=	minimal inhibition concentration
GRAS	=	generally recognized as safe
IBD	=	Inflammatory bowel disease
IgG	=	immunoglobulin G
TNF- α	=	tumor necrosis factor- α
TLR4	=	Toll-like receptor 4
LPS	=	lipopolysaccharides
PRR	=	pattern recognition receptor
NF- κ B	=	nuclear factor kappa B
IL	=	interleukin
rRNA	=	ribosomal RNA
PCR	=	polymerase chain reaction
Rep-PCR	=	repetitive element-based PCR
MRS	=	deMan Rogosa Sharp
NSS	=	normal saline solution
VA	=	vancomycin
ATCC	=	American type culture collection
MMRS	=	modified MRS broth
MH	=	Mueller Hinton agar
BHI	=	brain heart infusion agar BHI
OD	=	optical density
DNPH	=	dinitrophenylhydrazine
EHEC	=	enterohemorrhagic <i>E. coli</i>
ETEC	=	enterotoxigenic <i>E. coli</i>
EPEC	=	enteropathogenic, <i>E. coli</i>
EIEC	=	enterinvasive <i>E. coli</i>

LIST OF ABBREVIATIONS (CONT.)

mM	=	millimole
mm	=	millimetre
hr	=	hour
min	=	minute
s	=	second
°C	=	degree Celsius
G	=	gravity
μl	=	microliter
ng	=	nanogram
nm	=	nanometer
μg	=	microgram
mg	=	milligram
ml	=	millilitre
SD	=	standard deviation
bps	=	base pairs
ELISA	=	enzyme-linked immunosorbent assay
PBS	=	phosphate-buffered saline
FBS	=	fetal bovine serum FBS
HRP		horseradish peroxidase
LCM	=	<i>Lactobacillus</i> conditioned media
LDM	=	<i>Lactobacillus</i> defined medium
BSA	=	bovine serum albumin
TMB	=	tetramethyl benzidine
dNTP	=	dideoxynucleotide tri-phosphate
RDP	=	Ribosomal Database Project
MEGA	=	Molecular Evolutionary Genetics Analysis
NJ	=	neighbour-joining
UPGMA	=	unweighted-pair group method with arithmetic
AP-1	=	activator protein-1
JNK	=	c-Jun- N-terminal kinase
Egr-1	=	early growth response 1