

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



E47249

INHIBITORY ACTIVITY OF LACTOBACILLUS AGAINST THE  
EXTENDED - SPECTRUM  $\beta$  - LACTAMASE PRODUCING  
UROPATHOGENIC ENTEROBACTERIACEAE

KRITTAPAK HORCHAROEN

MASTER OF SCIENCE

IN MICROBIOLOGY

THE GRADUATE SCHOOL

CHIANG MAI UNIVERSITY

MAY 2010

600254184

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**KRITTAPAK HORCHAROEN**

**A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN  
PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF  
MASTER OF SCIENCE  
IN MICROBIOLOGY**

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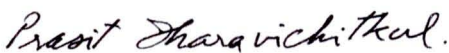
THIS THESIS HAS BEEN APPROVED  
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EXAMINING COMMITTEE



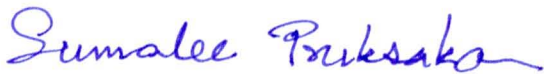
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14 May 2010

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## ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my advisor, Dr. Siriwoot Sookkhee, for his invaluable guidance, continuous discussion, helpful consultation, talent supervisions which made me learn so many things about life besides scientific knowledge and constant encouragement in laboratory works.

I am grateful to my coadvisors, Assoc. Prof. Prasit Tharavichitkul and Asst. Prof. Dr. Sumalee Pruksakorn for their kindnesses. I wish to express my great gratitude to the external thesis examination committee, Asst. Prof. Dr. Rith Watthanachaiyingcharoen.

I would also like to thanks to members of Microbiology Section, Central Diagnostic Laboratory, Maharaj Nakorn Chiang Mai Hospital to all successions and helpings.

Besides, I am greatly indebted to all my teachers at the Department of Microbiology, Faculty of Medicine, Chiang Mai University for their guidance, contribution of knowledge and sincere teaching. My thankfulness is also to all scientists, officers and all dear friends for their friendships and encouragements.

Finally, I own to eternal gratitude to my parents and my young brother for their support, understanding, patience, devoting, kindness and help which have been invaluable and inspired me to reach my goal.

Krittapak Horcharoen

<b>Thesis Title</b>	Inhibitory Activity of <i>Lactobacillus</i> Against the Extended - spectrum $\beta$ - lactamase Producing Uropathogenic Enterobacteriaceae	
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## ABSTRACT

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The present study was to determine the inhibitory effects of the potent lactobacilli toward the growth of extended - spectrum  $\beta$  - lactamase (ESBL) producing uropathogenic Enterobacteriaceae isolates and their  $\beta$  - lactamase activities. Among 172 *Lactobacillus* isolated from vagina of goats and foods, 2 isolates, namely L541 and LSS exhibited the strongest antimicrobial activities against 5 tested standard and 4 uropathogenic strains. According to API - 50 CHL kit, they were identified to be *Lactobacillus plantarum*1 and *Lactobacillus pentosus*, respectively. They produced too small amounts of lactic acid and hydrogen peroxide. The highest

quantity of their crude extracted bacteriocins could be precipitated by 40% saturation of ammonium sulfate. It may be suggested that their antimicrobial activities were caused from these extracted proteins. Among 101 isolates of multidrug resistant Enterobacteriaceae uropathogens, only 2 isolates of *Escherichia coli* and 2 isolates of *Klebsiella pneumoniae* which produced ESBL and also possessed the highest minimal inhibitory concentration to ceftazidime were selected. They significantly exhibited the  $\beta$  - lactamase activity after determined with the spectrophotometric nitrocefin assay. Furthermore, the above potent lactobacilli demonstrated the antimicrobial activity against various ESBL producing uropathogens. The growth inhibitions of 4 selected ESBL producing isolates could be demonstrated after cultured with these above lactobacilli or with the extracted bacteriocins. However, these bacteriocins showed the slightly reduction of the  $\beta$  - lactamase activity after mixed with these ESBL containing periplasmic proteins. It was concluded that lactobacilli and their bacteriocins could completely inhibit the growth of ESBL producing uropathogens but could interrupt in some degree of  $\beta$  - lactamase activity.

ชื่อเรื่องวิทยานิพนธ์

ฤทธิ์ยับยั้งของแลกโตบาซิลลัสต่อแบคทีเรียในวงส์  
เอนเทอโรแบคทีเรียซอี สายพันธุ์ก่อโรคในระบบทางเดิน  
ปัสสาวะ ที่ผลิตเอนไซม์บีตา - แลกแคมเอส ชนิดฤทธิ์ขยาย

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กรรมการ

## บทคัดย่อ

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การศึกษานี้มีวัตถุประสงค์เพื่อตรวจหาฤทธิ์ยับยั้งของแลกโตบาซิลลัส ต่อการเจริญของ  
แบคทีเรียในวงส์เอนเทอโรแบคทีเรียซอีสายพันธุ์ก่อโรคในระบบทางเดินปัสสาวะ ที่ผลิตเอนไซม์  
บีตา - แลกแคมเอส ชนิดฤทธิ์ขยาย และต่อประสิทธิภาพของเอนไซม์ บีตา - แลกแคมเอส ในจำนวน  
แลกโตบาซิลลัสทั้งสิ้น 172 ไอโซเลทที่แยกได้จากช่องคลอดและอาหารชนิดต่างๆ พบว่า  
แลกโตบาซิลลัส 2 ไอโซเลท คือ ไอโซเลท L541 และ LSS แสดงฤทธิ์ต้านเชื้อจุลินทรีย์ได้ดีที่สุดต่อ  
เชื้อทดสอบสายพันธุ์มาตรฐาน 5 สายพันธุ์และเชื้อก่อโรคในระบบทางเดินปัสสาวะ 4 สายพันธุ์  
เชื้อเหล่านี้ถูกจำแนกเป็นแลกโตบาซิลลัส แพลนท์ารัม และ แลกโตบาซิลลัส เพนโดซัส ตามลำดับ  
ด้วยชุดตรวจสอบ API - 50 CHL เชื้อนี้ผลิตกรดแลคติกและไฮโดรเจนเปอร์ออกไซด์ในปริมาณ  
น้อย ส่วนแบคทีเรียโอซินสกัดหาปริมาณที่สูงสามารถตกตะกอนได้ด้วยแอมโมเนียม ซัลเฟต  
ด้วยร้อยละ 40 อาจกล่าวได้ว่าฤทธิ์ต้านจุลินทรีย์นี้เป็นผลมาจากโปรตีนที่สกัดได้ ในจำนวนแบคทีเรีย

E47249

ในวงส์เอนเทอโรแบคทีเรียซิทีก่อโรคในระบบทางเดินปัสสาวะและดื้อยาหลายชนิด จำนวน 101 ไอโซเลทนั้นมีเพียง 2 ไอโซเลท ของเชื้อเอชเชอริเชีย โคไล และ 2 ไอโซเลทของเชื้อเครปเชียล่า นิวโมเนีย ซึ่งผลิตเอนไซม์บีตา - แลกแตมสชนิดฤทธิ์ขยาย และแสดงค่าความเข้มข้นที่ยับยั้งเชื้อได้น้อยที่สุดของยาเซฟตาซิมสูงสุด ได้ถูกคัดเลือกไว้ เชื้อเหล่านี้แสดงประสิทธิภาพของเอนไซม์บีตา - แลกแตมสได้อย่างมีนัยสำคัญภายหลังทดสอบด้วยวิธีสเปกโตรโฟโตเมตริก ในโครเซฟิน นอกจากนี้แลคโตบาซิลลัสสายพันธุ์ที่มีฤทธิ์ด้านจุลชีพเหล่านี้ แสดงฤทธิ์ด้านจุลชีพต่อแบคทีเรียก่อโรคในระบบทางเดินปัสสาวะ สายพันธุ์ที่ผลิตเอนไซม์บีตา - แลกแตมส ชนิดฤทธิ์ขยาย การยับยั้งการเจริญของเชื้อก่อโรคทั้ง 4 ไอโซเลทนี้ สามารถตรวจพบภายหลังบ่มเพาะด้วยแลคโตบาซิลลัส หรือด้วยแบคทีเรียโอซินที่สกัดได้ อย่างไรก็ตามแบคทีเรียเหล่านี้แสดงการลดลงเพียงเล็กน้อยของประสิทธิภาพของเอนไซม์บีตา - แลกแตมสภายหลังผสมร่วมกับโปรตีนจากเพอร์พลาสมที่มีเอนไซม์บีตา - แลกแตมสของเชื้อเหล่านี้ สรุปได้ว่าแลคโตบาซิลลัส และแบคทีเรียโอซินที่สกัดได้สามารถยับยั้งการเจริญของเชื้อก่อโรคในระบบทางเดินปัสสาวะ สายพันธุ์ที่ผลิตเอนไซม์บีตา - แลกแตมส ชนิดฤทธิ์ขยายได้อย่างสมบูรณ์ แต่สามารถรบกวนประสิทธิภาพของเอนไซม์บีตา - แลกแตมสได้บางส่วน

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## ABBREVIATIONS AND SYMBOLS

%	Percentage
°C	Degree celsius
µg	Microgram
µl	Microlitre
A.D.	Anno domini
AR	Analytical
BU	Bacteriocin unit
CFU	Colony forming unit
CO <sub>2</sub>	Carbon dioxide
e.g.	Exempli gratia (for example)
ESBL	Extended spectrum β-lactamases
<i>et al.</i>	et alii (and colleagues)
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
hrs.	Hours
i.e.	id est (that is)
kDa	Kilodalton
MIC	Minimal inhibitory concentration
min	Minute
ml	Millilitre
mm	Millimetre

nm	Nanometre
PBS	Phosphate buffer saline
pH	power of Hydronium
rpm	Revolution per minute
sec	Second
SDS	Sodium dodecyl sulfate
UPEC	Uropathogenic <i>Escherichia coli</i>
UTI	Urinary tract infection