INHIBITORY ACTIVITY OF LACTOBACILLUS AGAINST THE EXTENDED - SPECTRUM β - LACTAMASE PRODUCING UROPATHOGENIC ENTEROBACTERIACEAE

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MASTER OF SCIENCE
IN MICROBIOLOGY

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ABSTRACT

The present study was to determine the inhibitory effects of the potent lactobacilli toward the growth of extended - spectrum β - lactamase (ESBL) producing uropathogenic Enterobacteriaceae isolates and their β - 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 β - 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 β - 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 β - lactamase activity.

ชื่อเรื่องวิทยานิพนธ์ ฤทธิ์ยับยั้งของแลคโตบาซิลลัสต่อแบคทีเรียในวงศ์เอนเทอโรแบคทีเรียซิอี สายพันธุ์ก่อโรคในระบบทางเดินปัสสาวะ ที่ผลิตเอนไซม์บีตา - แลกแตเมส ชนิดฤทธิ์ขยาย

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บทคัดย่อ

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อตรวจหาฤทธิ์ยับยั้งของแลคโตบาซิลัส ต่อการเจริญของแบคทีเรียในวงศ์เอนเทอโรแบคทีเรียซิอีสายพันธุ์ก่อโรคในระบบทางเดินปัสสาวะ ที่ผลิตเอนไซม์บีตา - แลกแตเมส ชนิดฤทธิ์ขยาย และต่อประสิทธิภาพของเอนไซม์ บีตา - แลกแตเมส ในจำนวนแลคโตบาซิลลัสทั้งสิ้น 172 ไอโซเลทที่แยกได้จากช่องคลอดแพะและอาหารชนิดต่างๆ พบว่าแลคโตบาซิลลัส 2 ไอโซเลท คือ ไอโซเลท L541 และ LSS แสดงฤทธิ์ต้านเชื้อจุลชีพได้ดีที่สุดต่อเชื้อทดสอบสายพันธุ์มาตรฐาน 5 สายพันธุ์และเชื้อก่อโรคในระบบทางเดินปัสสาวะ 4 สายพันธุ์ เชื้อเหล่านี้ถูกจำแนกเป็นแลคโตบาซิลลัส แพลนทารัม และ แลคโตบาซิลลัส เพนโตซัส ตามลำดับด้วยชุดตรวจสอบ API - 50 CHL เชื้อนี้ผลิตกรดแลคติกและไฮโดรเจนเปอร์ออกไซด์ในปริมาณน้อย ส่วนแบคเทอริโอซินสกัดหยาบปริมาณที่สูงสามารถตกตะกอนได้ด้วยแอมโมเนียม ซัลเฟตอิ่มตัวร้อยละ 40 อาจกล่าวได้ว่าฤทธิ์ต้านจุลชีพนี้เป็นผลมาจากโปรตีนที่สกัดได้ ในจำนวนแบคทีเรีย

ในวงศ์เอนเทอโรแบคทีเรียซีอีที่ก่อโรคในระบบทางเดินปัสสาวะและดื้อยาหลายชนิด จำนวน 101 ไอโซเลทนั้นมีเพียง 2 ไอโซเลท ของเชื้อเอชเชอริเชีย โคไล และ 2 ไอโซเลทของเชื้อเครปเซียล่า นิวโมนิอี ซึ่งผลิตเอนไซม์บีตา - แลกแตเมสชนิดฤทธิ์ขยาย และแสดงค่าความเข้มข้นที่ยับยั้งเชื้อได้น้อยที่สุดของยาเซฟตาซิดิมสูงสุด ได้ถูกคัดเลือกไว้ เชื้อเหล่านี้แสดงประสิทธิภาพของเอนไซม์บีตา - แลกแตเมสได้อย่างมีนัยสำคัญภายหลังทดสอบด้วยวิธีสเปกโตรโฟโตเมทริก ไนโตรเซฟิน นอกจากนี้แลคโตบาซิลลัสสายพันธุ์ที่มีฤทธิ์ต้านจุลชีพเหล่านี้ แสดงฤทธิ์ต้านจุลชีพต่อแบคทีเรียก่อโรคในระบบทางเดินปัสสาวะ สายพันธุ์ที่ผลิตเอนไซม์บีตา - แลกแตเมส ชนิดฤทธิ์ขยาย การยับยั้งการเจริญของเชื้อก่อโรคทั้ง 4 ไอโซเลทนี้ สามารถตรวจพบภายหลังบ่มเพาะด้วยแลกโตบาซิลลัสหรือด้วยแบคเทอริโอซินที่สกัดได้ อย่างไรก็ตามแบคเทอริซินเหล่านี้แสดงการลดลงเพียงเล็กน้อยของประสิทธิภาพของเอนไซม์บีตา - แลกแตเมสภายหลังผสมร่วมกับโปรตีนจากเพอริพลาสมที่มีเอนไซม์บีตา - แลกแตเมสของเชื้อเหล่านี้ สรุปได้ว่าแลคโตบาซิลลัส และแบคเทอริโอซินที่สกัดได้สามารถยับยั้งการเจริญของเชื้อก่อโรคในระบบทางเดินปัสสาวะ สายพันธุ์ที่ผลิตเอนไซม์บีตา - แลกแตเมส ชนิดฤทธิ์ขยายได้อย่างสมบูรณ์ แต่สามารถรบกวนประสิทธิภาพของเอนไซม์บีตา - แลกแตเมสได้บางส่วน

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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_2	Carbon dioxide
e.g.	Exempli gratia (for example)
ESBL	Extended spectrum β-lactamases
et al.	et alii (and colleagues)
H_2O_2	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 *Escherichia coli*
UTI	Urinary tract infection