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GLUTAMATE DECARBOXYLASE, AMYLASE AND PROTEINASE  
FROM SELECTED *LACTOBACILLUS* AND *ENTEROCOCCUS* STRAINS

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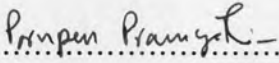
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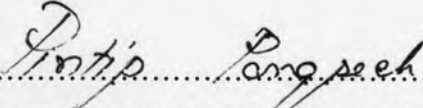
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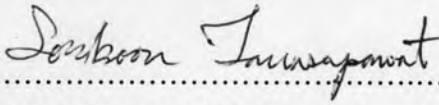
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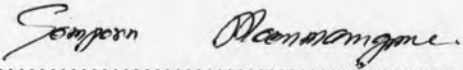
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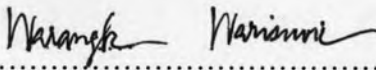
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ศิริพรรณ สุขคนธสิงห์ : กลูตาเมตดีคาร์บอกซีเลส อะมัยเลส และโปรตีนเนส จากแลคโตบาซิลลัส และเอนเทอโรคอคคัสสายพันธุ์ที่คัดเลือกได้ (GLUTAMATE DECARBOXYLASE, AMYLASE AND PROTEINASE FROM SELECTED *LACTOBACILLUS* AND *ENTEROCOCCUS* STRAINS) อ. ที่ปรึกษา : รศ.ดร.สมบุญ ธนาศุภวัฒน์, อ.ที่ปรึกษา ร่วม : Professor Kohei Oda Ph. D., 130 หน้า.

การคัดกรองแบคทีเรียกรดแลคติก ที่มีความสามารถในการสร้างเอนไซม์กลูตาเมตดีคาร์บอกซีเลส อะมัยเลส และ/หรือ โปรตีนเนส พบว่าจากแบคทีเรียกรดแลคติกจำนวนทั้งสิ้น 116 สายพันธุ์ที่แยกได้จากแหล่งต่างๆในประเทศไทย มีแบคทีเรียที่สามารถผลิตกรดแกมมาอะมิโนบิวทีริก (กาบา) รูปร่างแท่งจำนวน 4 สายพันธุ์ แบคทีเรียรูปร่างแท่งที่ย่อยแป้งจำนวน 2 สายพันธุ์ และรูปร่างกลมเป็นสายโซ่จำนวน 3 สายพันธุ์ รวมทั้งแบคทีเรียรูปร่างแท่งที่ย่อยโปรตีนได้จำนวน 2 สายพันธุ์ ผลการศึกษา ลักษณะทางพีโนไทป์ ลักษณะอนุกรมวิธานเคมีร่วมกับการวิเคราะห์ลำดับนิวคลีโอไทด์ของ *rpoA*, *pheS* และ 16SrDNA การวิเคราะห์แผนภาพไฟโลจีนติกส์ และการวิเคราะห์ความคล้ายคลึงกันของ ดีเอ็นเอ (DNA-DNA similarity) สามารถพิสูจน์เอกลักษณ์แบคทีเรียที่ผลิตกรดแกมมาอะมิโนบิวทีริก สายพันธุ์ LSF8-13 ได้เป็น *Lactobacillus brevis* FS73-1, SEA62-2 และ SR11-2 เป็น *L. plantarum* แบคทีเรียที่ย่อยแป้งสายพันธุ์ SB2-3 และ U3-1 เป็น *L. plantarum* FP 15-1 และ N2-1A เป็นแบคทีเรียสปีชีส์ใหม่ในสกุล *Enterococcus* และ N12-9 เป็น *E. gallinarum* โดยได้เสนอให้สายพันธุ์ FP 15-1 ซึ่งแยกได้จากใบเมี่ยงหมักเป็น *E. camelliae* sp. nov. แบคทีเรียที่ย่อยโปรตีนสายพันธุ์ SMC1 และ SCR1 เป็น *E. faecalis* and *L. sakei* ตามลำดับ นอกจากนี้ได้ทำการพิสูจน์เอกลักษณ์ และศึกษาอนุกรมวิธานของแบคทีเรียรูปร่างแท่งสายพันธุ์ L13 ซึ่งแยกได้จากผักดองพื้นบ้านของญี่ปุ่น และพบว่าแบคทีเรียเป็นสปีชีส์ใหม่ ในสกุล *Lactobacillus*

การศึกษาเปรียบเทียบยีน *gadB* ที่ทำหน้าที่ควบคุมการสังเคราะห์เอนไซม์กลูตาเมตดีคาร์บอกซีเลส (GAD) ของสายพันธุ์ L13, LSF 8-13, FS73-1, SEA62-2 และ SR11-2 โดยใช้ไพรเมอร์ที่ออกแบบจากข้อมูลการวิเคราะห์จีโนมของ *L. brevis* ATCC 367 และ *L. plantarum* WCFS1 ร่วมกับเทคนิค TAIL-PCR พบว่ายีน *gadB* ของ L13 มีขนาด 1437 เบส ถอดรหัสได้เป็นกรดอะมิโนขนาด 479 อะมิโน มีความคล้ายคลึงกับเอนไซม์ชนิดเดียวกันของ *L. brevis* ATCC 367, *L. plantarum* WCFS1 และ LSF 8-13 คิดเป็น 82.2, 51.5 และ 51.3 เปอร์เซ็นต์ตามลำดับ เมื่อทำการโคลนยีนที่แยกได้จาก L13 และ LSF 8-13 เข้าสู่พลาสมิดพาหะชนิด pQE70 และทำการกระตุ้นการแสดงออกของยีนใน *Escherichia coli* JM109 พบว่าสามารถกระตุ้นการผลิต และทำเอนไซม์ให้บริสุทธิ์โดยการผ่านสารสกัดเซลล์เข้าสู่คอลัมน์ Ni<sup>2+</sup>-HiTrap เอนไซม์บริสุทธิ์ที่ได้จาก L13 สามารถเร่งปฏิกิริยาการผลิตสารกาบาโดยมีความต้องการโคเอนไซม์ชนิด PLP ขณะที่เอนไซม์บริสุทธิ์จาก LSF 8-13 ไม่สามารถเร่งปฏิกิริยาการผลิตสารกาบาได้ เอนไซม์บริสุทธิ์ทั้งสองมีขนาดประมาณ 59 กิโลดาลตัน โดยวิธี SDS-PAGE ทำการวิเคราะห์เปรียบเทียบลำดับกรดอะมิโนและโครงสร้างสามมิติ การศึกษาครั้งนี้ประสบความสำเร็จในการแยก โคลน และกระตุ้นการแสดงออกของยีนกลูตาเมตดีคาร์บอกซีเลสจากแบคทีเรียกรดแลคติกสปีชีส์ใหม่สายพันธุ์ L13

สาขาวิชาเภสัชเคมีและผลิตภัณฑ์ธรรมชาติ ลายมือชื่อนิลิต.....ศิริพรรณ สุขคนธสิงห์.....

ปีการศึกษา 2549

ลายมือชื่ออาจารย์ที่ปรึกษา.....



# # 4676973933: MAJOR PHARMACEUTICAL CHEMISTRY AND NATURAL PRODUCTS

KEY WORD: GABA / GLUTAMATE DECARBOXYLASE / AMYLASE / PROTEINASE / LACTIC ACID BACTERIA

SIRAPAN SUKONTASING : GLUTAMATE DECARBOXYLASE, AMYLASE, AND PROTEINASE FROM SELECTED *LACTOBACILLUS* AND *ENTEROCOCCUS* STRAINS THESIS ADVISOR : ASSOC. PROF. SOMBOON TANASUPAWAT Ph.D., THESIS COADVISOR : PROF. KOHEI ODA Ph. D., 130 pp.

Totally 116 strains of lactic acid bacteria isolated from various sources in Thailand were screened for glutamate decarboxylase, amylase, and/or proteinase abilities. Four rod-shaped of  $\gamma$ -aminobutyric acid (GABA) producing bacteria, two rod-shaped and three cocci in chains of starch hydrolyzing bacteria including two rod-shaped of proteinase producing bacteria were isolated. On the basis of the phenotypic characteristics, *rpoA*, *pheS* and 16S rDNA sequencing, phylogenetic analysis, and DNA-DNA similarity, the GABA producing strains, LSF8-13 was identified as *Lactobacillus brevis*, FS73-1, SEA62-2 and SR11-2 were identified as *L. plantarum*. The rod-shaped starch hydrolyzing strains SB2-3 and U3-1 were identified as *L. plantarum*. The starch hydrolyzing cocci in chains, FP 15-1 and N2-1A were the novel species in *Enterococcus* and N12-9 was *E. gallinarum*. The strain FP15-1 isolated from fermented tea leaves is proposed as *Enterococcus camelliae* sp. nov. The proteinase producing strains, SMC1 and SCR1 were *E. faecalis* and *L. sakei* respectively. A high GABA producing lactobacilli L13 isolated from a traditional Japanese pickle (senmaizuke) was polyphasic studied. This strain is belonged to a new species in *Lactobacillus*

Glutamate decarboxylase (GAD) encoding gene (*gadB*) of the strains L13, LSF8-13, FS73-1, SEA62-2 and SR11-2 were subjected to be comparatively studied. The core fragments of *gadB* were isolated from the isolates, using primers based on highly conserved regions of predicted *gadB* from genomic sequence of *L. brevis* ATCC 367 and *L. plantarum* WCFS1. A full-length *gadB* of the strain L13 was subsequently isolated by TAIL-PCR method. Nucleotide sequence analysis of L13 *gadB* revealed that the open reading frame (ORF) consisted of 1437 bases and encoded a protein of 479 amino acid residues. The deduced amino acid showed 82.2, 51.5, and 51.3 % identity to the predicted GAD of *L. brevis* ATCC 367, *L. plantarum* WCFS1 and that of the isolated LSF 8-13 *gadB*, respectively. The isolated *gadB* of the strain L13 and LSF8-13 was constructed in an expression vector pQE70, cloned and expressed in *E. coli* JM109. The expressed GADs in crude extract of the transformed *E. coli* were purified by Ni<sup>2+</sup>-HiTrap chelating HP affinity column. The purified GAD showed a single protein band on SDS-PAGE. The molecular weight of the purified L13 and LSF8-13 GADs were approximately 59 kDa. The recombinant GAD of L13 showed GAD activity significantly depended on a present of PLP in enzyme reaction while that of LSF8-13 had no activity. Deduced amino acids and 3D structures were comparatively analyzed. Addressed here, a novel glutamate decarboxylase gene from the novel species L13 was successfully identified, cloned, and expressed.

Field of study Pharmaceutical Chemistry  
and Natural Products  
Academic year 2006

Student's signature.....*Sirapan S.*

Advisor's signature.....*Somboon Tanasupawat.*

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Dedicated to my passed away parents without whose painstaking labors during my childhood this work would never have been done.



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## LIST OF ABBREVIATIONS AND SYMBOLS

3D	three dimensional
ATCC	American Type Culture Collection, Manassas, USA
BSA	bovine serum albumin
BLAST	Basic Local Alignment Search Tool
bp	base pair
DDBJ	DNA data bank of Japan
DNA	deoxynucleic acid
GABA	$\gamma$ -aminobutyric acid
GAD	Glutamate decarboxylase
<i>gadB</i>	Glutamate decarboxylase encoding gene
kb	kilobases
kDa	kilo Dalton
kg	kilogram
LAB	Lactic acid bacteria
mg	milligram
ml	milliliter
MW	molecular weight
NCBI	National Center for Bioinformation
OD	optical density
ORF	open reading frame
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
rDNA	ribosomal deoxynucleic acid
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
Tris	tris(hydroxymethyl)aminomethane
$\lambda$	wavelength of light