

การจำแนกและลักษณะสมบัติของ cDNAs ของโปรตีนที่เกี่ยวข้องกับภูมิคุ้มกัน
จากเม็ดเลือดของกุ้งกุลาดำ *Penaeus monodon*
โดยเทคนิค Expressed Sequence Tags

นางสาว เปรมฤทัย สุพรรณกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

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**IDENTIFICATION AND CHARACTERIZATION OF cDNAs ENCODING
IMMUNE-RELATED PROTEINS IN THE HAEMOCYTE OF BLACK
TIGER SHRIMP *Penaeus monodon* BY EXPRESSED SEQUENCE TAGS**

Miss Premruethai Supungul

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for the Degree of Doctor of Philosophy in Biochemistry**

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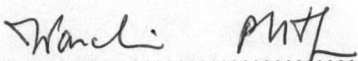
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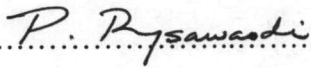
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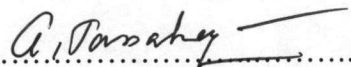
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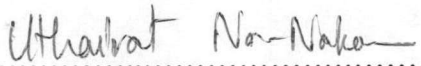
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ทำการสร้างห้องสมุด cDNA จาก mRNA ของกุ้งปกติ และกุ้งที่ติดเชื้อ *Vibrio harveyi* เพื่อแยกหายีนที่เกี่ยวข้องกับระบบภูมิคุ้มกันของกุ้งกุลาดำ (*Penaeus monodon*) โดยอาศัยเทคนิค Expressed Sequence Tags (ESTs) พบโคลนจากห้องสมุด cDNA ของกุ้งปกติ และของกุ้งติดเชื้อ จำนวน 1.4×10^6 และ 2.5×10^5 โคลน ตามลำดับ จากนั้นทำการหาลำดับนิวคลีโอไทด์โดยการสุ่มเลือกโคลนจำนวน 615 โคลน จากห้องสมุด cDNA ของกุ้งปกติ และ 447 โคลน จากห้องสมุด cDNA ของกุ้งติดเชื้อ นำผลที่ได้ไปเปรียบเทียบกับยีนที่รายงานแล้วใน GenBank โดยใช้โปรแกรม Blastn และ Blastx (NCBI Advanced Blast Search) พบว่า โคลนจำนวน 308 โคลน (50.1%) จากห้องสมุด cDNA ของกุ้งปกติ และ 215 โคลน (48.1%) จากห้องสมุดของกุ้งติดเชื้อมีลำดับนิวคลีโอไทด์คล้ายกับยีนที่รายงานแล้วใน GenBank โดยเป็นยีนที่แตกต่างกัน 288 ยีน ยีนในกลุ่มที่เกี่ยวข้องกับภูมิคุ้มกันพบทั้งสิ้น 34 ชนิด ได้แก่ ยีนในระบบโปรตีนออกซิเดส ยีนในระบบการแข็งตัวของเลือด ยีนของสารต้านจุลชีพ ตัวช่วยยังโปรติเอส และโปรตีนฮีตช็อก เป็นต้น ซึ่งยีนของสารต้านจุลชีพพบมากที่สุด โดยแอนติไลโปพอลิแซคคาไรด์ฟเลตเตอร์ พบมากเมื่อกุ้งมีการติดเชื้อ นอกจากนี้ พบยีนที่ครอบคลุมของโปรตีน 6 ชนิด คือ แอนติไลโปพอลิแซคคาไรด์ฟเลตเตอร์ ฟิเนียดิน ตัวช่วยยังโปรติเอส ครัสติน โปรตีนฮีตช็อก 10 และ ไซโทโซลิกซูเปอร์ออกไซด์ดีสมิวเทส

จากการศึกษาการแสดงออกของยีนในระบบภูมิคุ้มกัน 8 ชนิด โดยใช้เทคนิค semi-quantitative RT-PCR วัดระดับของ mRNA ในเลือดกุ้งปกติและติดเชื้อแบคทีเรีย ที่เวลา 3, 6, 12, 24 และ 48 ชั่วโมง ภายหลังการติดเชื้อแบคทีเรีย *Vibrio* พบการเพิ่มขึ้นอย่างมีนัยสำคัญ ($p < 0.05$) ของยีนแอนติไลโปพอลิแซคคาไรด์ฟเลตเตอร์ โปรตีนฮีตช็อก 90 และ ไลโซไซม์ และการลดลงอย่างมีนัยสำคัญ ($p < 0.05$) ของยีนฟิเนียดิน และครัสติน แต่ไม่พบการเปลี่ยนแปลงของยีนของตัวช่วยยังโปรติเอส โปรตีนออกซิเดส และ โปรตีนฮีตช็อก 70

ภาควิชา.....ชีวเคมี.....
 สาขาวิชา.....ชีวเคมี.....
 ปีการศึกษา.....2545.....

ลายมือชื่อนิสิต.....เปรมฤทัย สุพรรณกุล.....
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 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....
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PREMRUETHAI SUPUNGUL: IDENTIFICATION AND CHARACTERIZATION OF cDNAs ENCODING IMMUNE-RELATED PROTEINS IN THE HAEMOCYTE OF BLACK TIGER SHRIMP *Penaeus monodon* BY EXPRESSED SEQUENCE TAGS. THESIS ADVISOR : ASSOC. PROF. ANCHALEE TASSANAKAJON, Ph.D. THESIS CO-ADVISOR : SIRAWUT KLINBUNGA, Ph.D. AND RATH PICHYANGKURA, Ph.D. 207 pp. ISBN 974-17-3304-6

Two cDNA libraries were constructed from haemocytes of healthy and *Vibrio harveyi* infected shrimp *Penaeus monodon* to identify genes associated with immunity by the Expressed Sequence Tags (ESTs) technique. The number of clones were approximately 1.4×10^6 in the normal library and 2.5×10^5 in the infected library. Six hundreds and fifteen clones of the normal library and 447 clones of the infected library were randomly picked and partially sequenced. These sequences were compared with the previous sequence data in the GenBank by using the Blastn and Blastx programs (NCBI Advanced Blast Search). Three hundreds and eight (50.1%) of the EST clones of the normal library and 215 (48.1%) EST clones of the infected library matched significantly with the deposited genes. Five hundreds and thirty-two matched EST clones of both libraries represent 288 different proteins. One hundred and fifteen clones (10.8% of total sequencing clones) representing 34 different genes were putative immune genes. These genes are composed of those coding for enzymes and proteins of clotting system and the prophenoloxidase system, antimicrobial peptides, serine proteinase inhibitors, and heat shock proteins. Of these immune genes, the antimicrobial molecules were the most abundant (44.3% of immune genes). Interestingly, the EST coding for putative anti-lipopolysaccharide factor (ALF), an anti-Gram negative bacterial protein was predominate in the *V. harveyi* infected library. In addition, full-length ESTs encoding 6 different proteins were isolated. These are ALF, penaeidin, serine proteinase inhibitor, crustin, heat shock protein 10 and cytosolic manganese superoxide dismutase.

A semiquantitative RT-PCR was used to examine expression level of 8 immune genes in response to bacterial infection. A time course of mRNA expression on total RNA from haemocytes of unchallenged and *Vibrio* challenged shrimps at 3, 6, 12, 24 and 48 hr post injection showed significant increase in the expression level of ALF, heat shock protein 90 and lysozyme ($p < 0.05$) but significant decrease of penaeidin and crustin ($p < 0.05$). No change in the expression level of serine proteinase inhibitor, prophenoloxidase and heat shock protein 70 was observed.

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LIST OF ABBREVIATIONS

ALF	anti-lipoplysaccharide factor
bp	base pair
°C	degree Celcius
DEPC	Diethylpyrocarbonate
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
DNA	deoxyribonucleic acid
EtBr	ethidium bromide
LPS	Lipopolysaccharide
HSP10	heat shock protein 10
HSP 70	heat shock protein 70
HSP 90	heat shock protein 90
M	Molar
ml	Millilitre
MT	metric ton
MgCl ₂	magnesium chloride
mg	Milligram
mM	Millimolar
ng	Nanogram
O.D.	optical density
PCR	polymerase chain reaction
pfu	Plaque forming unit
proPO	Prophenoloxidase
proppA	prophenoloxidase activating enzyme
RNA	Ribonucleotide

RT	Reverse transcription
SPI	serine proteinae inhibitor
μg	Microgram
μl	Microlitre
μM	Micromolar