

การแยกและลักษณะสมบัติของยีนที่เกี่ยวข้องกับการพัฒนารังไข่ในกุ้งกุลาดำ

*Penaeus monodon*



นางสาว กาญจนา สิทธิขันแก้ว

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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ISOLATION AND CHARACTERIZATION OF GENE INVOLVING OVARIAN  
DEVELOPMENT OF THE BLACK TIGER SHRIMP *Penaeus monodon*

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A Thesis Submitted in Partial Fulfillment of the Requirements  
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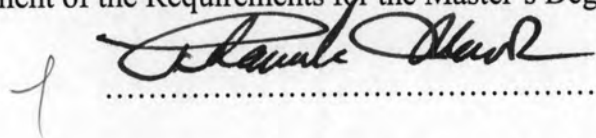
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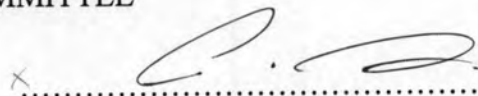
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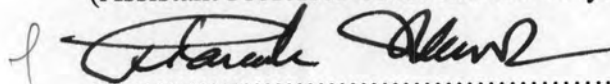
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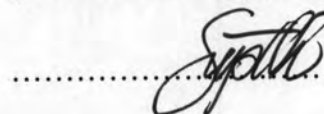
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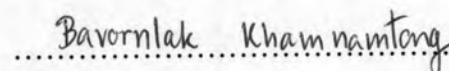
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กาญจนา สิทธิชันแก้ว การแยกและลักษณะสมบัติของยีนที่เกี่ยวข้องกับการพัฒนารังไข่ในกุ้งกุลาดำ *Penaeus monodon* (ISOLATION AND CHARACTERIZATION OF GENE INVOLVING OVARIAN DEVELOPMENT OF THE GIANT TIGER SHRIMP *Penaeus monodon*) อ. ที่ปรึกษา : ศ.ดร. เปี่ยมศักดิ์ เมณะเศวต, อ. ที่ปรึกษาร่วม : ดร. ศิราวุธ กลิ่นนุหงา 159 หน้า.

ทำการศึกษาลักษณะการแสดงออกของยีนในรังไข่ ( $N = 6$ ) และอณฑะ ( $N = 6$ ) ของพ่อ-แม่พันธุ์กุ้งกุลาดำ (*Penaeus monodon*) จำนวน 158 ยีนด้วยเทคนิค RT-PCR โดยออกแบบไพรเมอร์จาก EST libraries ของเม็ดเลือด (85 คู่ไพรเมอร์) รังไข่ (71 คู่ไพรเมอร์) และอณฑะ (2 คู่ไพรเมอร์) พบว่าสามารถให้ผลิตภัณฑ์พีซีอาร์จำนวน 111 ยีน ซึ่งมียีนที่แสดงออกในรังไข่แต่ไม่แสดงออกในอณฑะของกุ้งกุลาดำระยะเต็มวัยจำนวน 9 ยีนประกอบด้วย *female sterile (PMFS)*, *ATP/GTP binding protein*, *adipose differentiation related protein (PMADRP)*, *broad complex Z4 isoform*, *ovarian lipoprotein receptor (PMOVL R)*, *carbonic anhydrate*, *aminopeptidase*, *Wolf hirschorn syndrome candidate 1 protein*, และ *proactivator polypeptide precursor* และเมื่อตรวจสอบการแสดงออกของยีนในเนื้อเยื่อต่างๆ ของกุ้งกุลาดำพ่อ-แม่พันธุ์พบว่ายีน *female sterile* และ *ovarian lipoprotein receptor* มีการแสดงออกที่จำเพาะต่อรังไข่เท่านั้น นอกจากนี้พบยีนที่มีการแสดงออกในรังไข่มากกว่าในอณฑะจำนวน 64 คู่ไพรเมอร์ ตัวอย่างเช่น *3-oxoacid CoA transferase*, *NADP- dependent leukotrien B4 12 hydroxy dehydrogenase (PMLTB4DH)*, *dolichyl diphosphooligocharide protein glycotransferase (PMDDPG)*, *asparaginy l tRNA synthetase (PMATRS)*, *aspartate amino transferase (PMAST)*, *endothelial cell growth factor I (PMECGFI)* และ *nuclear autoantigenic sperm protein (PMNASP)*.

จากการตรวจสอบพอลิมอร์ฟิซึมของผลิตภัณฑ์ RT-PCR ของแต่ละยีน จำนวน 22 ยีนด้วยวิธี SSCP พบว่า ยีน *PMOVL P*, *PMNASP* and *tetrasparinD 107* มีพอลิมอร์ฟิซึมสูง โดยมีรูปแบบ SSCP ที่แตกต่างกันในตัวอย่างแต่ละตัว ซึ่งมีความเป็นไปได้ที่จะทำการศึกษาถึง SNP ที่มีความสัมพันธ์กับระดับการแสดงออกของยีนเหล่านี้ สำหรับยีน *asparaginy l tRNA synthetase (PMATRS)* พบว่า กุ้งกุลาดำที่มีค่าดัชนีรังไข่มีค่าเท่ากับ 1.90 - 2.13 เปอร์เซ็นต์ มีรูปแบบ SSCP ของยีนเพิ่มขึ้น เมื่อเปรียบเทียบกับกุ้งกุลาดำที่มีค่าดัชนีรังไข่มีค่าเท่ากับ 0.65 - 1.43 เปอร์เซ็นต์ ซึ่งอาจบ่งชี้ถึงความสำคัญของ *PMATRS* ต่อการเพิ่มขึ้นของโปรตีนอย่างรวดเร็วในระหว่างการสร้าง vitellogenin ของรังไข่ นอกจากนี้ยังพบความแตกต่างของรูปแบบ SSCP ระหว่างกุ้งเพศผู้และกุ้งเพศเมีย จากยีน *TATA binding protein associated factor9* ซึ่งอาจนำไปพัฒนาเป็นเครื่องหมายโมเลกุลที่มีความจำเพาะต่อเพศในกุ้งกุลาดำต่อไป

จากการหาลำดับนิวคลีโอไทด์ที่สมบูรณ์ของยีนต่างๆ จำนวน 9 ยีนพบว่าสามารถหาลำดับนิวคลีโอไทด์ที่สมบูรณ์ของยีนได้จำนวน 9 ยีน ประกอบด้วย *3-oxoacid CoA transferase*, *ATP/GTP binding protein*, *PMFS*, *PMADRP*, *PM*, *PMDDPG*, *PMATRS*, *PMAST*, *PMECGFI* และ *PMNASP* และ ลำดับนิวคลีโอไทด์บางส่วน (3315 นิวคลีโอไทด์) และส่วน 3' UTR ที่สมบูรณ์ (119 นิวคลีโอไทด์) ของ *PMOVL R* ซึ่งจะได้ทำการศึกษาค้นคว้าและหน้าที่ของยีนดังกล่าวต่อไป

5-HT เป็นสารที่สามารถควบคุมการปลดปล่อย neuropeptide hormones จากต่อม sinus ซึ่งจะมีผลต่อการพัฒนาของรังไข่ในกุ้ง เมื่อทำการฉีด 5-HT (50 ไมโครกรัม/น้ำหนักกุ้ง 1 กรัม) และตรวจสอบการแสดงออกของยีนต่างๆ ในรังไข่ ของกุ้งกุลาดำวัยรุ่นเพศเมีย พบว่า 5-HT สามารถกระตุ้นการแสดงออกของยีน *PMFS* และ *PMOVL P* อย่างมีนัยสำคัญทางสถิติ ( $P < 0.05$ ) เมื่อทำการฉีด 5-HT เพียง 1 ครั้ง แต่จะไม่กระตุ้นการแสดงออกของยีนดังกล่าวเมื่อทำการฉีดซ้ำ ( $P > 0.05$ ) ในขณะที่ 5-HT สามารถกระตุ้นการแสดงออกของยีน *PMADRP*, *PMNASP*, *PMAST*, *PMDDPG* และ *3-oxoacid CoA transferase* เมื่อทำการฉีด 5-HT จำนวน 1 ครั้ง ( $P < 0.05$ ) และ 2 ครั้ง ( $P < 0.05$ )

ลายมือชื่อนิสิต.....กาญจนา สิทธิชันแก้ว  
สาขาวิชา.....เทคโนโลยีชีวภาพ.....ลายมือชื่ออาจารย์ที่ปรึกษา.....  
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KEY WORD : *Penaeus monodon* / GIANT TIGER SHRIMP / OVARIAN DEVELOPMENT / RT-PCR

KANCHANA SITTIKHANKEAW: ISOLATION AND CHARACTERIZATION OF GENE INVOLVING OVARIAN DEVELOPMENT OF THE GIANT TIGER SHRIMP *Penaeus monodon*. THESIS ADVISOR: PROF. PIAMSAK MENASVETA, Ph. D. THESIS COADVISOR: SIRAWUT KLINBUNGA, Ph. D., 159 pp.

RT-PCR were carried out to examine the expression patterns of functionally important genes in ovaries ( $N = 6$ ) and testes ( $N = 6$ ) of the giant tiger shrimp (*Penaeus monodon*). A total of 158 pairs of primers were designed from EST of hemocyte (85 pairs), ovaries (71 pairs) and testes (2 pairs). Of these, 110 primers provided the positive amplification product. Nine gene homologues including *female sterile* (PMFS), *ATP/GTP binding protein*, *adipose differentiation related protein* (PMADRP), *broad complex Z4 isoform*, *ovarian lipoprotein receptor* (PMOVLRL), *carbonic anhydrase*, *aminopeptidase*, *Wolf hirschhorn syndrome candidate 1 protein* and *proactivator polypeptide precursor* were only expressed in ovaries but not testes of *P. monodon* broodstock where homologues of *female sterile* and *ovarian lipoprotein receptor* were ovarian-specific. Sixty-four gene homologues for example, *3-oxoacid CoA transferase*, *NADP-dependent leukotriene B4 12 hydroxy dehydrogenase* (PMLTB4DH), *dolichyl diphosphooligocharide protein glycotransferase* (PMDDPG), *asparaginyl tRNA synthetase* (PMATRS), *aspartate amino transferase* (PMAST), *endothelial cell growth factor 1* (PMECGFI) and *nuclear autoantigenic sperm protein* (PMNASP) were preferentially expressed in ovaries than testes of *P. monodon* broodstock.

SSCP analysis was applied for examination of polymorphism of the amplified RT-PCR product of 22 gene homologues across different individuals of *P. monodon*. PMOVLRL, PMNASP and *tetrasparinD 107* were highly polymorphic suggesting the possibility to study correlations between SNP of these gene homologues and expression levels (or phenotypes) of the corresponding genes in *P. monodon*. *Asparaginyl tRNA synthetase* was also polymorphic and additional forms of this transcript were found in the female shrimp possessing the GSI of 1.90 - 2.13 compared with those having the GSI between 0.65 - 1.43%. This indicated that *asparaginyl tRNA synthetase* (PMATRS) is required for rapid protein synthesis during vitellogenesis. A fixed SSCP pattern of *TATA binding protein associated factor 9* was found in ovaries and testes of *P. monodon* suggesting its sex-specific isotypes in *P. monodon*.

The full length cDNA of 9 gene homologues including *3-oxoacid CoA transferase*, *ATP/GTP binding protein*, PMFS, PMADRP, PMLTB4DH, PMDDPG, PMATRS, PMAST, PMECGFI and PMNASP were successfully characterized and reported for the first time. The partial ORF (3318 bp), the complete 3' UTR (116 bp) and the polyA tail of PMOVLRL were also isolated. Functional analysis of these gene homologues will be further studied.

5-HT modulates the release of neuropeptide hormones from the sinus gland of shrimp and its effect on ovarian maturation of shrimp has been proposed. A time course effect of 5-HT (single or double injection of 50  $\mu\text{g} \cdot \text{g}^{-1}$  of the body weight of 5-HT) on expression of PMFS, PMADRP, PMNASP, PMOVLRL, PMAST, PMDDPG and *3-oxoacid CoA transferase* in ovaries of juvenile *P. monodon* were examined using semiquantitative RT-PCR. Results indicate that 5-HT potentially stimulated the expression levels of all investigated genes ( $P < 0.05$ ). PMFS and PMOVLRL did not require the repeat injection of 5-HT as the second injection adverse the positive effect of the first injection. Nevertheless, repeat injection of 5-HT extended its effects on the expression level of PMADRP, PMNASP, *3-oxoacid CoA transferase*, PMDDPG and PMAST.

Student's signature..... Kanchana Sittikhankew

Field of study.....Biotechnology.....Advisor's signature.....

Academic year.....2006.....Co-advisor's signature.....



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**LIST OF ABBREVIATIONS**

bp	base pair
°C	degree celcius
DEPC	dethylpyrocarbonate
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
DNA	deoxyribonucleic acid
HCl	hydrochloric acid
IPTG	isopropyl-thiogalactoside
Kb	kilobase
M	molar
MgCl <sub>2</sub>	magnesium chloride
mg	mlligram
ml	mlilitre
mM	mllimolar
ng	nanogram
OD	optical density
PCR	polymerase chain reaction



RNA	ribonucleic acid
RNase A	ribonuclease A
rpm	revolution per minute
RT	reverse transcription
SDS	sodium dodecyl sulfate
Tris	tris (hydroxyl methyl) aminomethane
$\mu\text{g}$	microgram
$\mu\text{l}$	microlitre
$\mu\text{M}$	micromolar
UV	ultraviolet