การตอบสนองต่อความเครียดในกุ้งกุลาดำ *Penaeus monodon* โดยการตรวจวัดฮีตช็อกโปรตีนและระดับน้ำตาลในเลือด

นางสาวกาญจนา ด้วงปันตา

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STRESS RESPONSE IN BLACK TIGER PRAWN *PENAEUS MONODON* BY DETECTING HEAT SHOCK PROTEINS AND BLOOD GLUCOSE LEVEL

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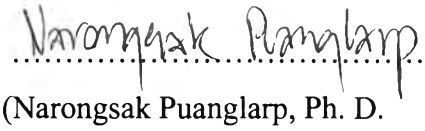

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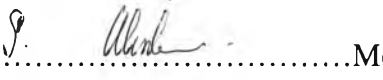

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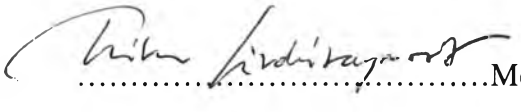

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กาญจนา ด้วงปันตา: การตอบสนองต่อความเครียดในกุ้งกุลาดำ *Penaeus monodon* โดยการตรวจวัดฮีตช็อกโปรตีนและระดับน้ำตาลในเลือด (STRESS RESPONSE IN BLACK TIGER PRAWN *PENAEUS MONODON* BY DETECTING HEAT SHOCK PROTEINS AND BLOOD GLUCOSE LEVEL) อาจารย์ที่ปรึกษา: ศ. ดร. เปี่ยมศักดิ์ เมนะเศวต, อาจารย์ที่ปรึกษาร่วม: ดร. ณรงค์ศักดิ์ พ่วงลาภ จำนวน 180 หน้า ISBN 974-17-6024-8

จากการศึกษาการตอบสนองต่อความเครียดของกุ้งกุลาดำที่อุณหภูมิต่างๆ พบว่าอุณหภูมิที่เป็น การกระตุ้นในระดับที่ไม่ทำอันตรายต่อชีวิตของกุ้งกุลาดำที่ขนาด 20 กรัม คือกระตุ้นที่ 15-35°C เป็นเวลา 6 ชั่วโมง พบว่ากุ้งมีชีวิตอยู่รอดได้มากกว่า 2 วัน และกุ้งที่กระตุ้นด้วยอุณหภูมิที่น้อยและสูงกว่านี้จะตายภายใน 10 และ 15 นาที หลังการกระตุ้นพบว่าระดับโปรตีนในน้ำเลือดจะมากขึ้น เมื่ออุณหภูมิเพิ่มขึ้นและลดลงเมื่อกระตุ้นด้วยความเย็นซึ่งผลนี้จะคล้ายกับระดับความเข้มข้นของน้ำตาลในน้ำเลือด และเมื่อศึกษาด้วย SDS-PAGE พบว่ามีแถบโปรตีนเกิดขึ้นแตกต่างกันหลายแถบทั้งในตัวอย่างที่ปกติและตัวอย่างที่ได้รับความเครียด อย่างไรก็ตามความแตกต่างดังกล่าวไม่สามารถหาข้อสรุปได้เนื่องจากปัญหาความไม่คงที่ของโปรตีนในตัวอย่าง และมีการปนเปื้อนของฮีโมไซยานินในตัวอย่าง และเมื่อนำเซลล์เม็ดเลือดของกุ้งในกลุ่มปกติ กลุ่มถูกกระตุ้นด้วยความเย็น และ กลุ่มถูกกระตุ้นด้วยความร้อนมาตรวจสอบด้วยวิธีเวสเทิร์นบลอทโดยใช้โมโนโคลนอลแอนติบอดีฮีตช็อกโปรตีน 70 พบแถบโปรตีนขนาด 76 กิโลดาลตัน ซึ่งในกลุ่มที่กระตุ้นด้วยอุณหภูมิจะมีแถบของโปรตีนเข้มกว่ากลุ่มควบคุม สำหรับในวิธีนี้ ฮีตช็อกโปรตีน 60 และ 90 ไม่สามารถตรวจวัดได้ การศึกษาการแสดงออกของยีนในเซลล์เม็ดเลือดของกุ้งที่มีการตอบสนองต่อการเปลี่ยนแปลงของอุณหภูมิโดยอาศัยวิธี RAP-PCR พบว่าจากผลการจับคู่ของแต่ละไพรเมอร์จำนวน 10 คู่ พบแถบดีเอ็นเอที่มีการแสดงออกในระดับที่ไม่เท่ากันจำนวน 7 แถบที่มีการแสดงออกมากขึ้นจำนวน 10 แถบและแสดงออกน้อยลงจำนวน 3 แถบ จากนั้นได้นำแถบดีเอ็นเอทั้งหมดไปโคลนและหาลำดับนิวคลีโอไทด์ พบว่ามี 3 แถบที่เหมือนกับยีนที่รู้หน้าที่ใน GenBank ส่วนผลการตรวจสอบการแสองออกของยีนจำนวน 8 ยีนประกอบด้วยเครื่องหมาย RAP-PCR (RAP12, 16, 22, และ 58), PO, HSP60, HSP70 และ HSP90 ต่อการตอบสนองต่ออีตช็อก และการติดเชื้อของ *Vibrio* ของกุ้งกุลาดำ พบว่าระดับการแสดงออกของ RAP12, RAP16, RAP22, RAP58, HSP70 และ HSP90 จะมากขึ้นต่อการตอบสนองด้วยการเหนี่ยวนำด้วยอุณหภูมิ สำหรับระดับการแสดงออกของยีน HSP60 ไม่สามารถตรวจพบ ในกลุ่มที่กระตุ้นด้วยวิบริโอไม่มีผลต่อการแสดงออกต่อยีนเหล่านี้ และการแสดงออกของยีน PO สามารถเหนี่ยวนำได้ในกลุ่มที่กระตุ้นด้วยวิบริโอ แต่ไม่แสดงออกในกลุ่มที่ได้รับการเหนี่ยวนำด้วยอุณหภูมิ โดยในการศึกษาครั้งนี้แสดงให้เห็นความสัมพันธ์ระหว่างอุณหภูมิการแสดงออกของยีนและความทนทานต่อเชื้อโรค

ลายมือชื่อนิสิต.....กาญจนา ด้วงปันตา.....

สาขาวิชา.....เทคโนโลยีชีวภาพ.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2547.....ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4372527723: MAJOR BIOTECHNLOGY
WORD: *Penaeus monodon* /protein/ glucose/RAP-PCR/RT-PCR/HSP

KANCHANA DOUNGPUNTA: STRESS RESPONSE IN BLACK TIGER PRAWN *PENAEUS MONODON* BY DETECTING HEAT SHOCK PROTEINS AND BLOOD GLUCOSE LEVEL. THESIS ADVISOR: PROF. PIAMSAK MENASVETA, Ph. D., THESIS CO-ADVISOR: NARONGSAK PUANGLARP, Ph. D., 180 pp. ISBN 974-17-6024-8.

The investigation on the stress response of *P. monodon* to various temperature revealed that the proper temperature for non-lethal thermal shock on 20 g shrimps ranged from 15 to 35°C for 6 h where shrimps survived for more than 2 days. Exposing to higher or lower temperatures of this range resulted in complete mortality within 10 and 15 min. After induction with non-lethal thermal shock, protein levels of haemolymph from induced shrimps raised significantly in corresponding to the level of the increase temperature in heat shock and decreased when exposed to cold shock. Similar results from the increase or decrease levels of plasma glucose concentration were obtained from thermal shock shrimps. The results on protein analysis of thermal-shock shrimps with and without vibrio infection using SDS-PAGE revealed several different protein bands between the unstressed and stressed samples. However, the results were not conclusive due to the un-consistency of the protein profiles between replications and the interference of haemocyanin in the samples. The Western blotting analysis of the haemocyte lysates from the shrimps from control, cold and heat shock experiments showed a considerably clear signal of cross reaction of anti-HSP70 monoclonal antibody and the proteins at 76 kDa. The increasing intensity of the band correlated to the level of thermal induction and time indicated that HSP70 was inducible by heat and cold condition and could be detected by cross-reaction with antibody raised with HSP70 from other organisms. The changes of HSP60 and HSP90 in thermal shock shrimps, however, were not detected by this method. The differential expressed genes in the haemocytes of the shrimps in response to heat shock were detected by RAP-PCR technique. From the result of amplification with 10 primer combinations, 7 DNA fragments were detected to display differentially between control and heat shock shrimps, 10 fragments were up regulated and 3 fragments were down regulated in corresponding to the heat shock temperatures. From the results of sequence comparison of those markers, 3 fragments showed significant similarity to some known genes in the GenBank. The expression levels of 8 stress-related genes including RAP-PCR markers (RAP12, 16, 22, and 58), PO, HSP60, HSP70 and HSP90 from heat-induced shrimps exposed to *Vibrio* were determined. The results showed that the transcription levels of RAP12, RAP16, RAP21, RAP58, HSP70, and HSP90 increased in responding to heat induction. The response of HSP60 gene to any of the inductions in this experiment was not detected. *Vibrio* treatment did not affect the expression level of those genes. The expression of PO gene was up-regulated by the *Vibrio* treatment but not by heat shock.

Student's signature.....Kanchana Doungpunta.....
Field of study.....Biotechnology.....Advisor's signature.....
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LIST OF ABBREVIATIONS

bp	base pair
°C	degree Celcius
BSA	Bovine serum albumin
CFU/ml	Colony forming unit/mililitre
DEPC	Diethylpyrocarbonate
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
DNA	deoxyribonucleic acid
EtBr	ethidium bromide
g	Gram
HSP	heat shock protein
kDa	Kilodalton
M	Molar
ml	Millilitre
MT	metric ton
$MgCl_2$	magnesium chloride
mg	Milligram
mM	Millimolar
MW	Molecular weight
ng	Nanogram
nm	Nanometre
O.D.	optical density
PBS	Phosphate buffer saline
PCR	polymerase chain reaction
PO	Phenoloxidase
RAP-PCR	RNA arbitrarily primed PCR
RNA	Ribonucleic acid
rpm	Revolution per minute

RT	Reverse transcription
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
PBS	Phosphate buffer saline
v/v	Volume by volume
w/v	Weight by volume
µg	Microgram
µl	Microlitre
µM	Micromolar