

ตัวบ่งชี้ทางชีวภาพในการตรวจสอบการตอบสนองต่อความเครียดในกิ้งกูดดำ *Penaeus monodon*



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**BIOMARKERS FOR DETERMINING THE STRESS RESPONSE**  
**IN BLACK TIGER SHRIMP *Penaeus monodon***

**Pol.Sub.Lt. Sansook Boonseub**

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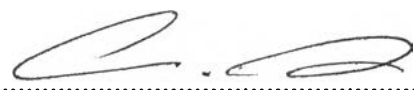
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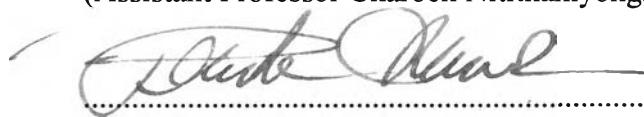
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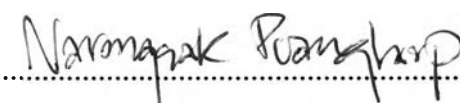
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จากการเพิ่มปริมาณชิ้นเมงกานีสซูปเปอร์ออกไซด์คิสมิวเตส และอาร์จินีน ไคเนสในเม็ดเลือดของกุ้ง  
 กุลาดำ พบว่าชิ้นเมงกานีสซูปเปอร์ออกไซด์คิสมิวเตสมีรูปแบบของยีนที่แตกต่างกัน 4 แบบ ส่วนยีนอาร์จินีน  
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 เมงกานีสซูปเปอร์ออกไซด์คิสมิวเตสสามารถพบได้ในปริมาณที่เท่าๆกันทั้งในเหงือก หัวใจ เม็ดเลือด ดับ  
 กล้ามเนื้อ และลำไส้ ในขณะที่พบปริมาณของอาร์จินีน ไคเนสมากที่สุด ในเหงือก รองลงมาคือหัวใจ เม็ดเลือด  
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 ดิก เคท 1 และไซโตโครซีคซินเปอร์ออกซิเดส ที่ตอบสนองต่อภาวะออกซิเดทีฟสเตรสจากการติดเชื้อไวรัส  
 ภาวะออสโมติกสเตรสจากการเปลี่ยนแปลงความเค็มของน้ำ และแซนดลิ่งสเตรสจากการรบกวนน้ำ โดยการกวน  
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 และ ฮีทช็อก 90 มีการแสดงออกของยีนลดลงเมื่ออยู่ในภาวะออกซิเดทีฟสเตรส ออสโมติกสเตรส และแซนดลิ่ง  
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 ของผลที่ชัดเจนนี้ต่อไป ในภาวะออสโมติกสเตรสการแสดงออกของยีนอาร์จินีน ไคเนสในเหงือกมีการ  
 แสดงออกของยีนลดลง ในขณะที่ภาวะแซนดลิ่งสเตรส พบว่าในเม็ดเลือดมีการแสดงออกของยีนอาร์จินีน  
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 ดลิ่งสเตรส การแสดงออกของยีนไซโตโครซีคซินเปอร์ออกซิเดส พบว่าไม่ถูกกระตุ้นในภาวะออสโมติกสเตรส  
 ส่วนในภาวะออกซิเดทีฟสเตรส และแซนดลิ่งสเตรส พบว่าระดับการแสดงออกของยีนไซโตโครซีคซินเปอร์ออก  
 ซิเดส ในเม็ดเลือดลดลง ในขณะที่ในเหงือกที่ภาวะออกซิเดทีฟสเตรสมีการแสดงออกของยีนนี้เพิ่มขึ้น ดังนั้น  
 จากงานวิจัยนี้สรุปได้ว่ายีนที่สามารถนำมาประยุกต์ใช้เป็นตัวบ่งชี้ทางชีวภาพในการตรวจสอบการตอบสนอง  
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**# # 4572561123: MAJOR BIOTECHNOLOGY**

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**SANSOOK BOONSEUB : BIOMARKERS FOR DETERMINING THE  
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THESIS ADVISOR. PROF. PIAMSAK MENASVETA, Ph.D., THESIS CO-  
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Mn-SOD gene and AK were amplified from haemocyte of *P.monodon*. Four novel variants of Mn-SOD were found whereas only one form of AK was observed. Tissue distribution and polymorphisms of Mn-SOD and AK of *P.monodon* were determined. The results indicated that the same level of Mn-SOD was clearly found in gill, heart, haemocyte, hepatopancreas, muscle and digestive tract while the highest level of AK was found in gill, followed by heart, haemocyte, hepatopancreas, muscle, and digestive tract, respectively. A number of stress responsive genes in *P.monodon*, including Mn-SOD, AK, HSP70, HSP90, DAD-I, and TPx were under investigation on their responses to oxidative stress causing by *vibrio* infection, osmotic stress inducing by salinity changes, and handling stress stimulating by disturbing water and exposing to air. Transcriptional levels of Mn-SOD in both haemocytes and gills of *P. monodon* were raised by the effect of oxidative, osmotic, and handling stress. The levels of HSP70 and HSP90 genes were reduced during oxidative, osmotic, and handling stress. These results are in contrast with the results reported in other studies. However, more study is needed to clarify the contradictory result. The level of AK gene expression in gill reduced during osmotic stress while up regulation of AK gene is observed in the haemocytes of handling stressed shrimps. The expression of DAD-I genes detected in gill of *P.monodon* was not induced by osmotic stress but repressed by oxidative and handling stresses. TPx gene was not induced by osmotic stress. The expression levels of TPx were decreased in haemocyte shrimps exposed to oxidative and handling stresses while it is raised in the gill of oxidative stressed shrimps. In conclusion, Mn-SOD, AK, and TPx can be further applied as biomarkers for determination of stresses in *P.monodon*.

Field of Study ..... Biotechnology.....Student's signature.....

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	<b>Page</b>
1.3.2 Osmotic stress.....	18
1.3.2.1. Homeostasis .....	19
1.3.2.2. Osmoregulation .....	19
1.3.2.3. Osmoregulation in crustaceans.....	20
1.3.2.4. Protein instability or denaturation by osmotic stress.....	21
1.3.3 Handling stress.....	22
1.4 Biomarker.....	23
1.4.1 Biomarker of exposure.....	23
1.4.2. Biomarker of effect.....	25
1.4.3 Biomarker of susceptibility.....	26
1.5 Application of biomarkers to Environmental Health and Risk Assessment.....	27
<b>CHAPTER II MATERIALS METHODS.....</b>	<b>29</b>
2.1 Animals .....	29
2.2 Preparation of <i>Vibrio harveyi</i> .....	29
2.3 Nucleic acid Extraction .....	30
2.3.1 RNA Extraction.....	30
2.4 First Strand cDNA Synthesis for Semi-quantitative RT-PCR.....	30
2.5 Determination of RNA and cDNA Concentration using Spectrophotometry and Electrophoresis.....	31
2.5.1 Spectrophotometry.....	31
2.5.2 Electrophoresis.....	31
2.6 Agarose Gel Electrophoresis.....	32
2.7 Primer Design.....	32
2.8 Amplification of Mn-SOD, Cu/Zn-SOD, and AK genes.....	34
2.9 Cloning and sequencing of target genes .....	34
2.9.1 Preparation of Competent Cells.....	34
2.9.2 Ligation of PCR Product.....	35
2.9.3 Transformation of Ligation Product.....	35



	<b>Page</b>
2.9.4 Detection Recombinant Clone by Colony PCR.....	36
2.9.5 Isolation of Recombinant Plasmid .....	36
2.9.6 Restriction enzyme digestion.....	38
2.9.7 Elution of DNA Fragments from Agarose Gel.....	38
2.9.8 DNA Sequencing.....	39
2.9.9 Data analysis.....	39
2.10 Single Stranded Conformational Polymorphism (SSCP) Analysis	39
2.10.1 Non-Denaturing Polyacrylamide Gel Electrophoresis.....	39
2.10.2 Preparation of Glass Plate.....	40
2.10.3 Preparation of SSCP Gel.....	40
2.10.4 Electrophoresis.....	40
2.10.5 Silver Staining.....	41
2.11 Conduction of stress treatment in <i>P.monodon</i> .....	42
2.11.1 Oxidative stress.....	42
2.11.2 Handle stress.....	42
2.11.2 Osmotic stress .....	42
2.12 Expression of Stress Response Genes Detected by Semi-quantitative RT-PCR .....	43
2.13 Statistic Analysis.....	45
<b>CHAPTER III RESULTS.....</b>	<b>46</b>
3.1 DNA Extraction.....	46
3.2 RNA Extraction .....	47
3.3 PCR amplification of Mn-SOD, Cu/Zn-SOD and AK.....	48
3.4 Polymorphisms of Mn-SOD and AK genes .....	50
3.4.1 Polymorphisms of Mn-SOD gene.....	50
3.4.2 Polymorphisms of AK gene .....	51
3.5 Variation of Mn-SOD DNA sequences .....	52
3.6 Expression of stress responsive genes in <i>P.monodon</i> .....	55
3.6.1 Optimization of Semi quantitative PCR condition .....	55

	<b>Page</b>
3.7 Survival rate of shrimps during osmotic stress.....	68
3.8 Expression level of stress responsive genes during osmotic stress.....	69
3.8.1 Expression Level of AK gene in gill .....	69
3.8.2 Expression Level of Mn-SOD gene in gill .....	70
3.8.3 Expression Level of HSP70 gene in gill .....	71
3.8.4 Expression Level of HSP90 gene in gill .....	71
3.8.5 Expression Level of DADI gene in gill .....	72
3.8.6 Expression Level of Thioredoxin peroxidase gene in gill .....	73
3.9 Survival rate of shrimps during oxidative stress.....	74
3.10 Expression level of stress responsive genes during oxidative Stress.....	75
3.10.1 Expression Level of Mn-SOD gene in haemocyte and gill .....	75
3.10.2 Expression Level of AK gene in haemocyte and gill.....	77
3.10.3 Expression Level of HSP70 gene in haemocyte and gill....	78
3.10.4 Expression Level of HSP90 gene in haemocyte and gill....	79
3.10.5 Expression Level of DADI gene in haemocyte and gill.....	80
3.10.6 Expression Level of Thioredoxin peroxidase gene in haemocyte and gill.....	81
3.11 Survival rate of shrimps during handling stress.....	82
3.12 Expression level of stress responsive genes during handling stress.....	83
3.12.1 Expression Level of Mn-SOD gene in haemocyte and gill...	83
3.12.2 Expression Level of AK gene in haemocyte and gill.....	85
3.12.3 Expression Level of HSP70 gene in haemocyte and gill.....	86
3.12.4 Expression Level of HSP90 gene in haemocyte and gill.....	87
3.12.5 Expression Level of DADI gene in haemocyte and gill.....	88
3.12.6 Expression Level of Thioredoxin peroxidase gene in haemocyte and gill.....	89

	<b>Page</b>
<b>CHAPTER IV DISCUSSIONS</b> .....	90
4.1 Tissue distribution and polymorphisms of Mn-SOD and AK .....	90
4.2 Expression of stress responsive genes in <i>P. monodon</i> .....	92
4.3 Potential genes as biomarkers for stress conditions in <i>P. monodon</i> .....	98
<b>CHAPTER V CONCLUSIONS</b> .....	100
<b>REFERENCES</b> .....	101
<b>APPENDICES</b> .....	128
Appendix A.....	129
Appendix B.....	130
Appendix C.....	144
Appendix D.....	175
<b>BIOGRAPHY</b> .....	176

## LIST OF TABLES

<b>Table</b>	<b>Page</b>
Table 1.1 Viruses affecting cultured and wild penaeid shrimp .....	7
Table 1.2 Biomarker of exposure.....	24
Table 1.3 Biomarker of effect.....	25
Table 1.4 Biomarker of exposure or effect .....	26
Table 2.1 Details of primers designed with the sequences from the DNA database of the GenBank and cDNA library of <i>P. monodon</i> .....	33
Table 3.1 Summary of the optimal conditions of semi-quantitative RT-PCR for determining the expression of stress responsive genes .....	56
Table 3.2 The ratio of AK gene and $\beta$ -actin gene in gill of <i>P.monodon</i> after stressed with salinity change at 15, 30, and 45 ppt for 2, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	69
Table 3.3 The ratio of Mn-SOD gene and $\beta$ -actin gene in gill of <i>P.monodon</i> after stressed with salinity change at 15, 30, and 45 ppt for 2, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	70
Table 3.4 The ratio of HSP70 gene and $\beta$ -actin gene in gill of <i>P.monodon</i> after stressed with salinity change at 15, 30, and 45 ppt for 2, 6, 12, 24, 48 and 72 h ( $N=3$ ).	71
Table 3.5 The ratio of HSP90 gene and $\beta$ -actin gene in gill of <i>P.monodon</i> after stressed with salinity change at 15, 30, and 45 ppt for 2, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	71
Table 3.6 The ratio of DADI gene and $\beta$ -actin gene in gill of <i>P.monodon</i> after stressed with salinity change at 15, 30, and 45 ppt for 2, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	72
Table 3.7 The ratio of Thioredoxin peroxidase gene and $\beta$ -actin gene in gill of <i>P.monodon</i> after stressed with salinity change at 15, 30, and 45 ppt for 2, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	73
Table 3.8 The ratio of Mn-SOD gene and $\beta$ -actin gene in haemocyte (A) and gill (B) of <i>P.monodon</i> after stressed with <i>Vibrio</i> exposure for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	76

	<b>Page</b>
Table 3.9 The ratio of AK gene and $\beta$ -actin gene in haemocyte (A) and gill (B) of <i>P.monodon</i> after stressed with <i>Vibrio</i> exposure for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	77
Table 3.10 The ratio of HSP70 gene and $\beta$ -actin gene in haemocyte (A) and gill (B) of <i>P.monodon</i> after stressed with <i>Vibrio</i> exposure for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	78
Table 3.11 The ratio of HSP90 gene and $\beta$ -actin gene in haemocyte (A) and gill (B) of <i>P.monodon</i> after stressed with <i>Vibrio</i> exposure for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	79
Table 3.12 The ratio of DADI gene and $\beta$ -actin gene in haemocyte (A) and gill (B) of <i>P.monodon</i> after stressed with <i>Vibrio</i> exposure for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	80
Table 3.13 The ratio of Thioredoxin peroxidase gene and $\beta$ -actin gene in haemocyte (A) and gill (B) of <i>P.monodon</i> after stressed with <i>Vibrio</i> exposure for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	81
Table 3.14 The ratio of Mn-SOD gene and $\beta$ -actin gene in haemocyte (A) and gill (B) of <i>P.monodon</i> after stressed with handling stress for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	84
Table 3.15 The ratio of AK gene and $\beta$ -actin gene in haemocyte (A) and gill (B) of <i>P.monodon</i> after stressed with handling stress for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	85
Table 3.16 The ratio of HSP70 gene and $\beta$ -actin gene in haemocyte and gill of <i>P.monodon</i> after stressed with handling stress for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	86
Table 3.17 The ratio of HSP90 gene and $\beta$ -actin gene in haemocyte (A) and gill (B) of <i>P.monodon</i> after stressed with handling stress for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	87
Table 3.18 The ratio of DADI gene and $\beta$ -actin gene in haemocyte (A) and gill (B) of <i>P.monodon</i> after stress with handling stress for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	88

	<b>Page</b>
Table 3.19 The ratio of Thioredoxin peroxidase gene and $\beta$ -actin gene in haemocyte and gill of <i>P.monodon</i> after stressed with handling stress for 0, 6, 12, 24, 48 and 72 h ( $N=3$ ).....	89
Table 4.1 Summary of the gene responses in <i>P.monodon</i> during stresses.....	99

## LIST OF FIGURES

<b>Figure</b>		<b>Page</b>
Figure 1.1	The external morphology of <i>Penaeus monodon</i> .....	4
Figure 1.2	The life cycle of <i>Penaeus monodon</i> .....	4
Figure 1.3	Schematic proceed of lipid peroxidation, chain reactions resulting in the formation of many lipid peroxide radicals.....	11
Figure 3.1	A 0.8% ethidium bromide strained agarose gel showing the quality of genomic DNA extracted from the muscle of <i>P.monodon</i> .....	46
Figure 3.2	A 1.2% ethidium bromide strained agarose gel showing the quality of RNA extracted from haemocyte and gill of <i>P.monodon</i> .....	47
Figure 3.3	PCR products of AK (a), Cu/Zn-SOD (b) and Mn-SOD genes (c) separated in 1.2% agarose gel.....	49
Figure 3.4	DNA sequence of Mn-SOD determined on 10% acrylamide gel by SSCP technique and stained with silver solution.....	50
Figure 3.5	Nucleotide sequence of AK determine on 10% acrylamide gel by SSCP technique and stained with silver solution.....	51
Figure 3.6	Multiple alignments of Mn-SOD DNA fragments of <i>P. monodon</i> .....	52
Figure 3.7	Multiple alignment of deduced amino acid sequence of Mn-SOD of <i>P. monodon</i> .....	53
Figure 3.8	Schematic representation of the structure of full length of Mn-SOD gene.....	53
Figure 3.9	Multiple alignment deduce amino acid of Mn-SOD of <i>P. monodon</i> retrieved from Genbank, PCR product amplified with Mn-SOD specific primer and EST library of <i>P.monodon</i> .....	54
Figure 3.10	DNA sequence of $\beta$ -actin gene. The highlight shows the position of Actin1 and Actin2 primer.....	57
Figure 3.11	PCR product of Mn-SOD amplification determined on 1.2% agarose gel and stained with Ethidium bromide.....	58

	<b>Page</b>
Figure 3.12 Relationship between PCR products of Mn-SOD gene amplified from haemocyte cell of <i>P.monodon</i> and various amount of template used in PCR reaction.....	58
Figure 3.13 PCR product of AK amplification determined on 1.2% agarose gel and stained with Ethidium bromide.....	59
Figure 3.14 Relationship between PCR products of AK gene amplified from haemocyte cell of <i>P.monodon</i> and various amount of template used in PCR reaction.....	59
Figure 3.15 PCR product of HSP70 amplification determined on 1.2% agarose gel and stained with Ethidium bromide.....	60
Figure 3.16 Relationship between PCR products of HSP70 gene amplified from haemocyte cell of <i>P.monodon</i> and amount of template used in PCR reaction.....	60
Figure 3.17 PCR product of HSP90 amplification determined on 1.2% agarose gel and stained with Ethidium bromide.....	61
Figure 3.18 Relationship between PCR products of HSP90 gene amplified from haemocyte cell of <i>P.monodon</i> and amount of template used in PCR reaction.....	61
Figure 3.19 PCR product of DADI amplification determined on 1.2% agarose gel and stained with Ethidium bromide.....	62
Figure 3.20 Relationship between PCR products of DADI gene amplified from haemocyte cell of <i>P.monodon</i> and various amount of template used in PCR reaction.....	63
Figure 3.21 PCR product of Thioredoxin peroxidase amplification determined on 1.2% agarose gel and stained with Ethidium bromide.....	64
Figure 3.22 Relationship between PCR products of Thioredoxin peroxidase gene amplified from haemocyte cell of <i>P.monodon</i> and various amount of template used in PCR reaction.....	65
Figure 3.23 PCR product of $\beta$ -actin amplification determined on 1.2% agarose gel and stained with Ethidium bromide.....	66



	<b>Page</b>
Figure 3.24 Relationship between PCR products of $\beta$ -actin gene amplified from haemocyte cell of <i>P.monodon</i> and various amount of template used in PCR reaction.....	67
Figure 3.25 Survival rate of shrimps stressed with salinity changes. Shrimps acclimated in seawater at 15 ppt were shocked with water at salinity of 0, 15, 30, 45, and 60 ppt.....	68
Figure 3.26 Survival rate of shrimp stressed with <i>V.harveyi</i> administration at $10^6$ CFU/ml.....	74
Figure 3.27 Survival rate of shrimp stressed by disturbing water for 5 minutes (4 times in every 30 minutes) and moving the shrimps out of water for 1 minute.....	82
Figure A.1 Map of the pGEM <sup>®</sup> -T easy vector and multiple cloning sites....	129
Figure A.2 Nucleotide sequence of Mn-SOD of <i>P. monodon</i> from cDNA library of <i>P.monodon</i> .....	137
Figure A.3 Nucleotide sequence of AK of <i>P. monodon</i> from cDNA library of <i>P.monodon</i> .....	142
Figure A.4 Alignment of nucleotide sequence of Cu/Zn product from DNA sequencing with cytochrome c oxidase subunit 3.....	143
Figure A.5 The expression level of AK gene transcripts in hemocyte of pre-stress shrimp and between control and stress with salinity change in comparison with $\beta$ -actin gene.....	144
Figure A.6 The expression level of AK gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with $\beta$ -actin gene.....	145
Figure A.7 The expression level of Mn-SOD gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with $\beta$ -actin gene.....	146
Figure A.8 The expression level of HSP70 gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with $\beta$ -actin gene.....	147

Figure A.9	The expression level of HSP90 gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with $\beta$ -actin gene.....	148
Figure A.10	The expression level of DADI gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with $\beta$ -actin gene.....	149
Figure A.11	The expression level of Thioredoxin peroxidase gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with $\beta$ -actin gene.....	150
Figure A.12a	The expression level of Mn-SOD gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comparison with $\beta$ -actin gene.....	151
Figure A.12b	The expression level of Mn-SOD gene transcripts in gill from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comparison with $\beta$ -actin gene.....	152
Figure A.13a	The expression level of AK gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comparison with $\beta$ -actin gene.....	153
Figure A.13b	The expression level of AK gene transcripts in gill from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comparison with $\beta$ -actin gene.....	154
Figure A.14a	The expression level of HSP70 gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comparison with $\beta$ -actin gene.....	155

Figure A.14b	The expression level of HSP70 gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comparison with $\beta$ -actin gene.....	156
Figure A.15a	The expression level of HSP90 gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comparison with $\beta$ -actin gene.....	157
Figure A.15b	The expression level of HSP90 gene transcripts in gill from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comparison with $\beta$ -actin gene.....	158
Figure A.16a	The expression level of DADI gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comparison with $\beta$ -actin gene.....	159
Figure A.16b	The expression level of DADI gene transcripts in gill from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comperison with $\beta$ -actin gene.....	160
Figure A.17a	The expression level of Thioredoxin peroxidase gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comparison with $\beta$ -actin gene.....	161
Figure A.17b	The expression level of Thioredoxin peroxidase gene transcripts in gill from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with <i>V.harveyi</i> exposure in comparison with $\beta$ -actin gene.....	162
Figure A.18a	The expression level of Mn-SOD gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene.....	163

	<b>Page</b>
Figure A.18b The expression level of Mn-SOD gene transcripts in gill from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene...	164
Figure A.19a The expression level of AK gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene.....	165
Figure A.19b The expression level of AK gene transcripts in gill from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene...	166
Figure A.20a The expression level of HSP70 gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene.....	167
Figure A.20b The expression level of HSP70 gene transcripts in gill from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene...	168
Figure A.21a The expression level of HSP90 gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene.....	169
Figure A.21b The expression level of HSP90 gene transcripts in gill from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene...	170
Figure A.22a The expression level of DADI gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene.....	171
Figure A.22b The expression level of DADI gene transcripts in gill from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene...	172

	<b>Page</b>
Figure A.23a The expression level of Thioredoxin peroxidase gene transcripts in hemocyte from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene.....	173
Figure A.23b The expression level of Thioredoxin peroxidase gene transcripts in gill from <i>P.monodon</i> of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with $\beta$ -actin gene.....	174

## LIST OF ABBREVIATIONS

bp	Base pair
°C	Degree Celsius
cDNA	Complementary deoxyribonucleic acid
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanosine triphosphate
dNTP	Deoxyribonucleotide triphosphate
dTTP	Deoxythymidine triphosphate
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetate
g	Gram
g	Gravity (multiples of, as in centrifugal field)
HCl	Hydrochloric acid
IPTG	Isopropyl-thiogalactoside
kb	Kilo base
LB	Luria-Bertani
M	Molar (mole per litres)
MgCl <sub>2</sub>	Magnesium chloride
mg	Milligram
ml	Milliliter
mM	Millimolar
ng	Nanogram
OD	Optical density
PCR	Polymerase chain reaction
RNase A	Ribonuclease A
rpm	Revolution per minute
SDS	Sodium dodecyl sulfate
SSCP	Single strand conformation polymorphism

**LIST OF ABBREVIATIONS (Cont.)**

T <sub>m</sub>	Temperature, melting
Tris	Tris (hydroxyl methyl) aminomethane
U	Unit
μg	Microgram
μl	Microlitre
μM	Micromolar
UV	Ultraviolet
v/v	Volume / volume (concentration)
w/v	Weight/ volume (concentration)
λ	Lambda