EXPRESSION OF IMMUNE-RELATED GENES AT DIFFERENT POST-LARVAE STAGES OF TIGER SHRIMP, *Penaeus monodon*

ZULAIKHA BINTI MAT DERIS

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DEDICATION

I dedicate this thesis to my late beloved mother, my father, sisters, brothers and family for all their supports and encouragement throughout my studies and also to all my beloved friends for all of their help and support.

Thank you

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Main Supervisor : Li Lian Wong, Ph.D

Co-Supervisor : Shumpei Iehata, Ph.D

Institute : Institute of Tropical Aquaculture and Research Fisheries (AKUATROP)

Acute Hepatopancreatic Necrosis Disease (AHPND), a disease caused by *Vibrio parahaemolyticus* (Vp_{AHPND}), results in mass mortalities worldwide in the Penaeid shrimp aquaculture industry, leading to severe financial losses. To understand the immunity response of *Penaeus monodon* post-larvae (PL) towards Vp_{AHPND} infection, the shrimp survivability, expression of immunity genes and presence of bacterial toxin genes were investigated and validated in three stages of *P. monodon* PL (PL15, PL30 and PL45). In a total of 20-hour time-course challenge test conducted for the PL stages that were exposed to 2.7×10^7 cfu ml^{-1} of Vp_{AHPND}, highest mortality was observed in PL 30 (81%), followed by PL15 (65%) and PL45 (1.67%). Immunity gene expression profiles further supported that PL30 was most susceptible towards Vp_{AHPND} infection compared to PL15 and PL45, with PL30 showing a 10.73 fold for Toll-like receptor (TLR), 4.67 fold for prophenoloxidase (proPO), 6.45 fold for lysozyme (lyso) and 3.21 fold for penaeidin (PEN). This might be caused by the different feeding regimes during culturing in order to mimic the aquaculture setting.The expressions of shrimp immunity genes of PL30 was initiated at 1 hour post infection (hpi) and peaked at both 16 hpi and 20 hpi, but sharply decreased at 18 hpi, highlighting the crucial involvement of these genes in the defence and recovery phases of the first-line defence

mechanisms of PLs when infected with Vp_{AHPND}. In the second part of this study, bacterial gene expression profiles suggested that toxR gene is a good indicator gene to detect the presence of *Vibrio* spp., while PIR A gene is exclusively used for the determination of Vp_{AHPND} pathogenicity effects on *P. monodon*. Overall, these findings provide novel insights into the immunity defense phases of different PL stages towards Vp_{AHPND} infection at transcription levels, in which PL30 was observed to be highly susceptible to Vp_{AHPND} carrying PIR A toxin genes.

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EKPRESI GEN KEIMUNAN PADA PERINGKAT PASCA LARVA YANG BERBEZA BAGI UDANG HARIMAU, *Penaeus monodon*

ZULAIKHA BINTI MAT DERIS

2019

Penyelia Utama : Li Lian Wong, Ph.D

Penyelia Bersama : Shumpei Iehata, Ph.D

Institut : Institut Akuakultur Tropika dan Penyelidikan Perikanan (AKUATROP)

Acute Hepatopancreatic Necrosis Disease (*AHPND*) adalah penyakit yang disebabkan oleh bakteria *Vibrio parahaemolyticus* (Vp_{AHPND}). *AHPND* telah menyebabkan kematian udang *Penaeid* dalam industri akuakultur di seluruh dunia, yang mengakibatkan kerugian yang sangat tinggi. Bagi memahami tindak balas keimunan pasca larva *Penaeus monodon* (PL) terhadap jangkitan Vp_{AHPND}, tahap kemandirian udang, ekspresi gen keimunan dan kehadiran toksin gen bakteria telah dikaji dan disahkan dalam tiga peringkat PL *P. monodon* (PL15, PL30 dan PL45). Ujian jangka masa selama 20 jam telah dijalankan. Setiap peringkat PL telah didedahkan kepada 2.7 x 10^7 *cfu* ml^{-1} Vp_{AHPND} dan kadar kematian tertinggi adalah pada PL 30 (81%), diikuti oleh PL15 (65%) dan PL45 (1.67%). Profil ekspresi gen keimunan menunjukkan PL30 adalah lebih mudah terdedah kepada kesan jangkitan Vp_{AHPND} berbanding PL15 dan PL45. PL30 menunjukkan ekspresi tertinggi dalam gandaan 10.73 bagi *Toll-like receptors* (TLR), 4.67 bagi *prophenoloxidase* (proPO), 6.45 bagi *lysozyme* (lyso) dan 3.21 untuk *penaeidin* (PEN). Keputusan ini, berkemungkinan disebabkan oleh faktor perbezaan pemakanan yang telah dijalankan bagi menyamai sistem akuakultur sebenar. Ekspresi gen keimunan udang dalam PL30 bermula pada 1 jam pasca jangkitan (*hour post infection*- hpi) dan memuncak pada 16 hpi dan 20 hpi, tetapi menurun secara mendadak pada 18 hpi. Ini menunjukan penglibatan yang penting oleh gen ini dalam

fasa pertahanan dan mekanisme pemulihan sebagai sistem pertahanan utama dalam PL apabila dijangkiti oleh Vp_{AHPND}. Profil ekspresi gen bakteria menunjukan bahawa gen toxR adalah penanda gen yang paling sesuai untuk mengesan kehadiran *Vibrio* spp. Manakala, gen PIR A digunakan secara eksklusif untuk menentukan kesan patogenik Vp_{AHPND} pada *P. monodon*. Secara keseluruhannya, penemuan dalam kajian ini memberikan gambaran yang baharu mengenai fasa rintangan keimunan pada peringkat PL yang berbeza terhadap jangkitan Vp_{AHPND} di peringkat transkripsi. PL30 didapati paling mudah dijangkiti oleh Vp_{AHPND} yang membawa gen toksik PIR A.