

THE IMMUNOSTIMULATORY ROLE OF
BACTERIAL HEAT SHOCK PROTEINS AND THEIR
EFFICACY IN PROTECTING THE WHITE-LEG
SHRIMP, *Litopenaeus vannamei* AGAINST VIBRIO-
INDUCED MORTALITY

SITI MUNIRAH BINTI RAZALI

MASTER OF SCIENCE
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**Thesis Submitted in Fulfillment of the Requirement for the Degree of Master
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Feeding bacteria encapsulated heat shock proteins (HSPs) represents a novel alternative to battle Vibriosis in aquaculture, an important disease caused by the bacterium *Vibrio*. The objectives of this study are to determine the effect of feeding bacterial HSPs, challenge against *V. harveyi* and also the mRNA encoding in *L. vannamei* larvae. Feeding YS2, a transformed *E. coli* containing plasmids that over-express prokaryotic DnaK, which corresponds to eukaryotic HSP70, is shown in this study to protect *L. vannamei* post-larvae against pathogenic *V. harveyi*, boosting survival more than 20% in a standardized challenge assay. Further enhancement occurred with L-arabinose induction, suggesting a role for HSP accretion in the protection of shrimp larvae against pathogenic *Vibrio*. YS2 does not contribute nutritionally to the larvae, as indicated by insignificant larvae increases in growth during feeding tests ($P > 0.05$). Incubation with YS2 over-producing DnaK enhanced the amount of crustin, a potent antimicrobial peptide for the Penaeid shrimp approximately 5.8 fold. Likewise, feeding of P3, an *E. coli* strain transformed with a plasmid over-expressing prokaryotic DnaK-DnaJ-grpE, which corresponds to eukaryotic HSP70-HSP40-HSP20 enhanced shrimp survival 41% in the presence of *V. harveyi*. Incubation of shrimp larvae with induced P3 increased crustin approximately 23.7 fold. The attenuation of pathogenic *Vibrio* may be associated with crustin synthesis.

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**PERANAN IMMUNOSTIMULATORI PROTEIN-PROTEIN RENJATAN
HABA BAKTERIA DAN KEBERKESANANNYA DI DALAM
PERLINDUNGAN UDANG PUTIH, *Litopenaeus vannamei* TERHADAP
MORTALITY YANG DIRANGSANG OLEH VIBRIO.**

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Memberi makan bakteria yang mengandungi heat shock proteins merupakan alternatif baru untuk melawan Vibriosis di dalam akuakultur, iaitu penyakit yang penting disebabkan oleh bakteria Vibrio. Objektif kajian ini adalah untuk mengenalpasti kesan pemakanan bakteria HSPs, cabaran melawan *V. harveyi* dan mRNA didalam larva *L.vannamei*. Memberi makan YS2, satu pengubahan E.coli yang mengandungi plasmids lebih-mengekspresi DnaK prokariot yang sepadan dengan eukariot HSP70, ditunjukkan dalam kajian ini untuk melindungi *L. vannamei* pasca larva terhadap patogen *V. harveyi*, meningkatkan survival lebih daripada 20% dalam ujian cabaran seragam. Peningkatan selanjutnya wujud dengan induksi L-arabinose, mencadangkan peranan penambahan HSP di dalam perlindungan larva udang terhadap patogenik vibrio.YS2 tidak menyumbang dari segi nutrisi terhadap larvae merujuk kepada peningkatan dalam pertumbuhan larval yang tidak signifikan semasa ujian pemberian makanan ($P>0.05$). Inkubasi dengan YS2 yang menghasilkan lebih DnaK meningkatkan jumlah crustin, rangkaian antimikrob yang berkesan kepada udang penid lebih kurang 5.8 kali ganda. Begitu juga dalam pemberian makanan P3 yang merupakan E.coli strain, diubah dengan plasmid lebih mengekspresi prokariot DnaK-DnaJ-grpE yang sepadan dengan eukariot HSP70-HSP60-HSP40 meningkatkan sebanyak 41% survival udang dengan kehadiran *V.harveyi*. Inkubasi larvae dengan P3 induksi meningkatkan crustin lebih kurang 23.7 kali ganda. Ini difahamkan bahawa pengurangan patogen Vibrio mungkin berkaitan dengan sintesis crustin.