

EFFECT OF THERMAL SHOCK ON HSP70
EXPRESSION AND INNATE PROTECTION
AGAINST VIBRIO INFECTION IN THE WHITE-
LEG SHRIMP *Litopenaeus vannamei* (BOONE, 1931)

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MASTER OF SCIENCE
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Abstract

Exposure to long-term hyperthermic stress disrupts the normal physiological processes of shrimp, and under severe conditions may decrease immune response towards disease and infection, resulting in mortality. Short-term heat stress, however, promotes bacterial and viral disease tolerance, possibly due to heat shock protein 70 (Hsp70) accumulation and/or immune enhancement. The expression of Hsp70, and the immune-related genes Prophenoloxidase (ProPO), Peroxinectin, Penaeidin, Crustin and Hemocyanin of the white-leg shrimp *Litopenaeus vannamei*, following a short-term hyperthermic stress was studied herein. A 30 min heat shock from 28°C to 34°C, 36°C and 38°C enhanced Hsp70 production in *L. vannamei* post-larvae (PL), with the Hsp70 mRNA increased respectively 1.4, 1.4 and 2.1 fold, in comparison to non-heated control animals. Immunoprobings of western blots and quantification by ELISA revealed that the increase in Hsp70 production correlated with Hsp70 mRNA expression, the latter verified by qRT-PCR. ProPO and Hemocyanin mRNA levels were induced approximately 10.0, 9.3 and 7.9 fold and 15.0, 5.6 and 2.6 fold, respectively whereas Peroxinectin and Crustin mRNA levels were unchanged after heat shock. Penaeidin mRNA was down regulated in all heat shock treatments. Challenge tests performed with different concentrations of *V. alginolitycus*, *V. parahaemolyticus* and *V. harveyi* revealed that *V. harveyi* was virulent at 1.0×10^7 cells/ml, resulting to 50% mortality 48 h after immersion. Conversely, *V. alginolitycus* and *V. parahaemolyticus* were not pathogenic in all concentrations applied in this study. A 30 min non-lethal heat shock from 28°C to 34°C, 36°C, 38°C did not enhance survival in the challenge test, indicating that shrimp resistance to pathogenic *V. harveyi* was neither afforded by Hsp70 accumulation nor immune-related gene expression following thermal shock.

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Abstrak

Pendedahan kepada tekanan hipertermik jangka masa panjang mengganggu proses fisiologi normal udang, dan di bawah keadaan yang melampau boleh menurunkan tindakbalas imun terhadap penyakit dan jangkitan, serta menyebabkan kematian. Walau bagaimanapun, tekanan haba jangka masa pendek meningkatkan toleransi terhadap penyakit bawaan bakteria dan virus, berkemungkinan disebabkan oleh pengumpulan heat shock protein 70 (Hsp70) dan / atau peningkatan imun. Ekspresi Hsp70, dan gen berkaitan dengan imunisasi Prophenoloxidase (ProPO), Peroxinectin, Penaeidin, Crustin dan Hemocyanin pada udang putih *Litopenaeus vannamei*, mengikut tekanan hipertermik jangka masa pendek telah dikaji. Renjatan haba selama 30 minit dari suhu 28°C ke 34°C, 36°C dan 38°C meningkatkan penghasilan Hsp70 di dalam pasca larva (PL) *L. vannamei*, dengan mRNA Hsp70 masing-masing meningkat sebanyak 1,4, 1,4 dan 2,1 kali ganda, berbanding dengan haiwan kawalan yang tidak dipanaskan. Immunoprobings western blots dan kuantifikasi oleh ELISA menunjukkan bahawa peningkatan dalam penghasilan Hsp70 berkait dengan ekspresi-mRNA Hsp70, yang seterusnya disahkan oleh qRT-PCR. Tahap ProPO dan mRNA Hemocyanin masing-masing ditingkatkan kira-kira 10.0, 9.3 dan 7.9 kali ganda dan 15.0, 5.6 dan 2.6 kali ganda, manakala tahap Peroxinectin dan mRNA Crustin tidak berubah selepas renjatan haba. mRNA penaeidin menurun dalam semua rawatan kejutan haba. Ujian cabaran menggunakan *V. parahaemolyticus*, *V. alginolyticus*, dan *V. harveyi* pada kepekatan yang berlainan menunjukkan bahawa *V.*

harveyi adalah virulen pada 1.0×10^7 sel / ml, dan mengakibatkan kematian sebanyak 50% selepas 48 jam rendaman. Sebaliknya, *V. alginolyticus* dan *V. parahaemolyticus* adalah tidak patogenik pada kepekatan yang diaplikasikan di dalam kajian ini. Kejutan haba selama 30 minit dari suhu 28°C kepada 34°C, 36°C, dan 38°C tidak menyebabkan peningkatan kemandirian dan ini menunjukkan bahawa daya ketahanan larva udang terhadap *V. harveyi* patogenik tidak bergantung pada pengumpulan Hsp70 atau ekspresi gen imun selepas renjatan haba.

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