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Feeding aquatic animals with bacterial encapsulated heat shock proteins (Hsps) is potentially a new method to combat Vibriosis, an important disease affecting aquatic animals used in aquaculture. In this study, food pellets containing *Escherichia coli* over-expressing either DnaK-DnaJ-GrpE, the prokaryotic equivalents of Hsp70-Hsp40-Hsp20 (pellet P3+), or only DnaK (pellet YS2+), were prepared and the viability of bacteria on the pellets was determined. Maintaining pellets at different temperatures for varying lengths of time reduced the number of live adhering *E. coli*, as did contact with seawater, demonstrating that storage and immersion adversely affected bacterial survival and attachment to pellets. Thus, freshly coated pellets were fed to *Penaeus vannamei* juveniles and protection against pathogenic *Vibrio harveyi* was determined weekly for 1 month, work that included verification of their immune status upon bacterial Hsp uptake.

Feeding *P. vannamei* with *E. coli* did not compromise survival and growth, indicating that the bacteria were not pathogenic to shrimp. Supplying *P. vannamei* with either pellets P3+ or YS2+ containing approximately 60 bacteria/g pellets boosted shrimp survival two-fold against *V. harveyi* suggesting that bacterial Hsps play a role in *Vibrio* tolerance. Pellets containing bacterial Hsps provided

protection for up to two weeks before loss of viability demonstrating that Hsps encapsulated by these bacteria enhanced shrimp resistance against *Vibrio* infection. Quantitation by qRT-PCR revealed that mRNAs encoded by the immune-related genes penaedin, Hsp70 and prophenoloxidase mRNA were significantly up-regulated in shrimp muscle fed with pellets P3+ or YS2+. Hemolymph crustin, Hsp70 and prophenoloxidase mRNA increased with feeding pellet P3+, while penaedin, peroxinectin and prophenoloxidase mRNA were raised in shrimp fed with pellet YS2+. Taken together, these results indicated that ingestion of bacterial Hsps, particularly DnaK, influences the immune status of juvenile shrimp by regulating immune related genes expression. Which immune related genes examined in this study enhanced shrimp tolerance to Vibrios is however unknown and further work is necessary to study how DnaK promotes tolerance. Findings from such studies may assist in the development of strategies to protect shrimp against disease during aquaculture.

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STATUS IMUN DAN TOLERANSI JUVENIL UDANG *Penaeus vannamei* TERHADAP VIBRIOSIS SELEPAS PEMBERIAN PROTEIN RENJATAN HABA BAKTERIA

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Penyuapan bakteria mengandungi heat shock proteins (Hsps) kepada organisma akuatik berpotensi sebagai kaedah baru untuk lawan Vibriosis, sejenis penyakit yang memberi kesan kepada organisma akuatik yang digunakan dalam akuakultur. Dalam kajian ini, makanan udang yang mengandungi bakteria *Escherichia coli* yang mengekspresi DnaK-DnaJ-GrpE yang bersamaan dengan Hsp70-Hsp40-Hsp20 (pelet P3+), atau DnaK sahaja (pelet YS2+) telah disediakan dan ketahanan bakteria pada pellet dikaji menunjukkan pelet yang disimpan di suhu yang berbeza dan yang direndam dalam air masin untuk jarak masa yang tertentu telah mengurangkan jumlah *E. coli* yang terdapat pada pelet. Oleh itu, pellet yang baru disediakan diberi kepada udang putih, Penaeus vannamei juvanil selama 1 bulan dan kajian untuk verifikasi status imunisasi terhadap pengambilan Hsps bakteria dilakukan.

Pemberian pelet yang mengandungi *E. coli* tidak menjejaskan kemandirian dan tumbesaran udang menunjukkan bakteria ini tidak patogen kepada udang. *P. vannamei* yang diberi makan pelets P3+ atau YS2+ (lebih kurang 60 bakteria/g pelet) mempunyai ketahanan terhadap *V. harveyi* sebanyak dua kali ganda daripada eksperimen kawalan menyarankan Hsps bakteria memainkan peranan dalam toleransi terhadap *Vibrio*. Pelet yang mengandungi Hsps bakteria efektik selama dua minggu dalam melindungi udang daripada *Vibrio*. Ini menunjukkan bakteria yang mengandungi Hsps meningkatkan

ketahanan udang terhadap jangkitan *Vibrio*. Kuantitasi dengan qRT-PCR menunjukkan mRNA penaedin, Hsp70 dan prophenoloxidase telah meningkat dalam tisu *P. vannamei* yang diberi makan dengan pelet P3+ atau YS2+ manakala dalam hemolimfa, mRNA crustin, Hsp70 dan prophenoloxidase telah meningkat dalam *P. vannamei* yang diberi makan pelet P3+ dan mRNA penaedin, peroxinectin dan prophenoloxidase meningkat dalam *P. vannamei* yang diberi makan pelet YS2+. Secara keseluruhan, hasil kajian ini menunjukkan penyuapan Hsps bakteria khususnya DnaK mempengaruhi status imunisasi udang dengan meregulasi gen berkait dengan imunisasi sepanjang eksperimen. Pada ketika ini, adalah sukar untuk menentukan gen imunisasi yang mana yang terlibat dalam meningkatkan perlindungan udang terhadap *Vibrios*. Penemuan daripada kajian sebegini boleh membantu dalam penemuan strategi baru untuk melindungi udang daripada penyakit semasa akuakultur.