PREVALENCE AND MOLECULAR CHARACTERIZATION OF *VIBRIO PARAHAEMOLYTICUS* ISOLATED FROM CULTURED TIGER PRAWNS (*PENAEUS MONODON*) FROM MALACCA

By

TUAN ZAINAZOR TUAN CHILEK

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master Science

June 2006

DEDICATION

This piece of work is dedicated to my lovely wife (Nani), who has always been by my side and given me the encouragement and support that carries me through my study period. Not forgetting my daughter (Fifi) and parents for their endless love to me.

Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Professor Son Radu, PhD

Faculty : Food Science and Technology

Food industries play an important role and contribute to economic activities in many countries worldwide. However, the frequencies of outbreak of food borne cases reported around the world related to this industry posed a significant public health issue. All countries including Malaysia have already developed their own food laws and food regulations to protect public health and to prevent food borne outbreaks. However, despite the strict controlled at every stages of food production, from farm to table, food borne outbreaks still occur. Tiger Prawn (*Penaeus monodon*) is synonymous with Malaysian farming industries. *Vibrio parahaemolyticus* is one of the major seafood-borne gastroenteritis-causing bacteria, frequently associated with consumption of improperly cooked seafood. Cultured tiger prawn farm at LKIM Merlimau, Malacca was identified for sampling in this study. The duration of sampling followed the whole cycle of tiger prawn cultivation

(until harvesting stage) involving two cycles (from 29 September 2003 to 29 April 2004). A total of sixty samples of cultured tiger prawn (25g/sample) and pond's water (25ml/sample) from both cycles were obtained. In our study, we found that most samples acquired were positive for *V. parahaemolyticus* (81.7%) when examined with specific-PCR (*toxR*). The presence of virulence gene, the *tdh* and/or *trh* genes have been considered the major virulence factor of the bacterium. In our study, none *tdh* gene was detected in all isolates. However, *trh* gene was detected in twenty-three isolates (38.3%) for both prawn and water samples. Temperature, pH and salinity play as an important factors in every stage of culturing tiger prawns. The optimum condition for those factors (temperature; 37^{o}C, pH; 7.8-8.6, salinity; 3%) were promoted the growth of *V. parahaemolyticus*. RAPD-PCR was used to generate polymorphic genomic fingerprints to determine of genetic relatedness among *V. parahaemolyticus* isolates. Two primers (OPAR3; 5'-CTTGAGTGGA-3'and OPAR8; 5'-GAGATGACGA-3'), out of the ten primers showed the best results and were selected for further study. Primer OPAR3 and OPAR8 produced 1 to 8 bands and 1 to 9 bands, respectively with amplified products ranging from 0.25 to 10.0 kilo base pairs. Seven groups of antibiotics namely, the Aminoglycosides, β-lactams, Cephalosporins, Glycopeptides, Macrolides, Quinolones, Tetracyclines and others were tested against all *V. parahaemolyticus* isolates. They were highly resistant to ampicillin (100%), penicillin (100%), cefuroxim (100%), teicoplanin (100%), erythromycin (98%), rifampicin (98%), trimethoprim

(98%) and streptomycin (96%), but sensitive to quinolones and tetracyclines groups of antibiotic. The antibiotic resistance profile patterns can be classified into four groups. In this study, MAR index range was between 0.40 – 0.60. Tiger prawn is a potential source of *V. parahaemolyticus*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMANTAUAN DAN PENCIRIAN MOLEKULAR BAGI *VIBRIO PARAHAEMOLYTICUS* YANG DIPENCILKAN DARIPADA UDANG HARIMAU (*PENAEUS MONODON*) YANG DITERNAK DI NEGERI MELAKA

Oleh

TUAN ZAINAZOR TUAN CHILEK

Jun 2006

Pengerusi : Profesor Son Radu, PhD

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Industri makanan memainkan peranan yang penting dan menyumbang kepada kegiatan ekonomi bagi kebanyakan negara. Pada masa kini, kejadian wabak yang berpunca daripada makanan sering dilaporkan di seluruh dunia yang melibatkan industri ini. Semua negara termasuk Malaysia, mereka telah membangunkan sistem undang-undang makanan dan peraturan-peraturan makanan mereka sendiri bagi melindungi kesihatan awam dan membenteras kejadian wabak. Walaupun kawalan ketat ke atas setiap peringkat pengeluaran makanan, dari ladang kepada pengguna dijalankan, namun ia tetap terjadi. Udang harimau berkait rapat dengan industri penternakan di Malaysia. *Vibrio parahaemolyticus* merupakan faktor utama kejadian keracunan makanan laut yang kebiasaannya berkait rapat dengan pengambilan makanan laut yang tidak dimasak sepenuhnya.

Ladang ternakan udang harimau di Negeri Melaka telah dipilih bagi kajian ini. Tempoh pensampelan melibatkan dua kitaran penternakan udang harimau iaitu bermula dari 29 hb. September 2003 sehingga 29 hb. April 2004. Sebanyak 60 sampel termasuk udang harimau (25g/sampel) dan air kolam ternakan (25ml/sampel) telah diambil sepanjang tempoh tersebut. Di dalam kajian ini didapati 81.7% daripada sampel menunjukan kehadiran *V. parahaemolyticus* apabila dianalisa menggunakan kaedah PCR (*toxR*). Kehadiran genetik yang berbahaya seperti *tdh* dan *trh* menunjukan bakteria tersebut adalah patogenik. Genetik *tdh* tidak dikesan di dalam semua pencilan bakteria bagi kajian ini. Walaubagaimanapun, genetik *trh* dikesan di dalam 23 pencilan (38.3%) yang diperolehi daripada sampel udang dan air. Suhu, pH dan kandungan garam merupakan faktor-faktor yang penting yang mempengaruhi penternakan udang harimau bagi setiap peringkat penternakan. Keadaan optimum bagi faktor tersebut (suhu; 37^{o}C, pH; 7.8-8.6, kandungan garam; 3%) akan membantu pertumbuhan *V. parahaemolyticus*. Kaedah RAPD-PCR telah digunakan bagi mendapatkan perkaitan genetik di antara pencilan *V. parahaemolyticus* yang diperolehi. Daripada 10 primer yang disaring, 2 primer (OPAR3; 5'-CTTGAGTGGA-3'dan OPAR8; 5'-GAGATGACGA-3') yang memberikan keputusan terbaik telah dipilih untuk kajian seterusnya. Primer OPAR3 dan OPAR8 masing-masing menghasilkan 1 hingga 8 band dan 1 hingga 9 band dengan saiz di antara 0.25 hingga 10.0 kb. Tujuh kumpulan antibiotik seperti Aminoglycosides, β-lactams, Cephalosporins, Glycopeptides, Macrolides,

Quinolones, Tetracyclines dan lain-lain telah digunakan. Pencilan *V. parahaemolyticus* yang diperolehi daripada ladang ternakan udang menunjukan kerintangan yang tinggi ke atas ampicillin (100%), penicillin (100%) , cefuroxim (100%), teicoplanin (100%), erythromycin (98%), rifampicin(98%), trimethoprim (98%) dan streptomycin (96%) tetapi sensitif kepada kumpulan antibiotik seperti quinolones dan tetracyclines. Melalui kajian ini, MAR indeks yang diperolehi adalah di antara 0.40 hingga 0.60. Udang harimau mempunyai potensi sebagai sumber bagi *V. parahaemolyticus*.

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I certify that an Examination Committee has met on 19th June 2006 to conduct the final examination of Tuan Zainazor Bin Tuan Chilek on his Master of Science thesis entitled "Prevalence and Molecular Characterization of *Vibrio parahaemolyticus* Isolated from Cultured Tiger Prawn (*Penaeus monodon*) from Malacca" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follow:

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DECLARATION

I hereby that the thesis based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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TUAN ZAINAZOR TUAN CHILEK

Date:

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