MOLECULAR CHARACTERIZATION OF *Vibrio parahaemolyticus* ISOLATED FROM THE COASTS OF PORT KLANG AND LUKUT LOCATED ALONG PENINSULAR MALAYSIA

By

GWENDELYNNE BULAN TANIL

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

February 2004

DEDICATION

To,

My loving parents, Tanil Baru and Molly Ting,
My inspiring sister and brother, Sue & Tim,
My true friend & companion, Seph

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

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Chairman : Professor Dr. Son Radu

Faculty : Food Science and Technology

Vibrio parahaemolyticus is a halophilic bacterium frequently involved in seafood-associated gastroenteritis. In this study, *V. parahaemolyticus* was isolated from 14 (36.8%) of 40 seawater samples taken from coastal areas of three fishing villages namely Kuala Lukut in Negri Sembilan, Morib and Port Klang in Selangor. A total of 21 strains were studied for their antibiotic resistance, the occurrence of plasmids, and the presence or absence of a regulatory gene (*toxR*), and virulence genes (*tdh* and *trh*), the molecular fingerprint by enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) and pulsed field gel electrophoresis (PFGE) analysis. This study has shown that all strains were multiple resistant to three or more of the fourteen antibiotics tested with the MAR indices ranging from 0.29 to 0.57. The high MAR indices indicate that the strains originated from high-risk sources. Most strains were resistant to penicillin

(100%), ampicillin (95.24%), carbenicillin (95.24%), erythromycin (95.24%) and bacitracin (71.43%). Some strains showed resistance to antibiotics such as cephalothin (28.57%), moxalactam (28.57%), kanamycin (19.05%), tetracycline (14.29%), nalidixic acid (9.52%), and gentamicin (9.52%). All strains were susceptible to streptomycin, norfloxacin and chloramphenicol. Seven strains harbored only one plasmid, 6 strains harbored two plasmids, and 8 strains had no plasmid. The plasmid size ranged from 2.6 to 35.8 MDa. All strains carried the *toxR* gene specific to *V. parahaemolyticus* while none had the *tdh* gene. However, one strain harbored the *trh* gene. Two molecular typing methods were used in this study to examine the genetic relatedness among the strains collected from different areas. In the analysis by the ERIC-PCR, primers ERIC 1R and ERIC 2 were used. In this analysis, 15 different ERIC profiles were observed, displaying a wide array of diversity among the strains; the strains could be differentiated into 2 clusters and 14 single isolates based on 70% similarity level of the dendogram constructed. In the PFGE method, *Sfi I* restriction enzyme was used. Cluster analysis of the PFGE profiles divided the strains into 4 clusters and 9 single isolates based on the 70% similarity level. However, this method suffered from substantial DNA degradation resulting in four untypeable strains. This study, demonstrated that the coastal seawater in Peninsular Malaysia is a potential source for pathogenic *V. parahaemolyticus* that can contaminate the seafood harvested from these areas.

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

PENCIRIAN MOLEKULAR BAKTERIA *Vibrio parahaemolyticus* YANG DIPENCILKAN DARIPADA PESISIRAN PANTAI PELABUHAN KLANG DAN KUALA LUKUT DI SEMENANJUNG MALAYSIA

Oleh

GWENDELYNNE BULAN TANIL

Februari 2004

Pengerusi : Professor Dr. Son Radu

Fakulti : Sains dan Teknologi Makanan

Vibrio parahaemolyticus merupakan bacteria halofilik yang sering dikaitkan dengan gastroenteritis yang berpunca dari makanan laut. Dalam kajian ini, *V. parahaemolyticus* telah dipencilkan daripada 14 (36.8%) dari 40 sampel air laut yang diperolehi dari tiga kawasan berlainan iaitu Kuala Lukut di Negeri Sembilan, Morib dan Pelabuhan Klang di Selangor. Sejumlah 21 pencilan dikaji untuk kerintangan terhadap antibiotik, kehadiran plasmid, kewujudan gen regulator *toxR* dang en-gen virulen *tdh* dan *trh*, serta pencirian ERIC-PCR dan analisis PFGE. Hasil kajian ini telah mendapati bahawa semua pencilan mempunyai kerintangan terhadap tiga atau lebih jenis antibiotik daripada empat belas jenis antibiotik yang digunakan dengan jukat nilai MAR dari 0.29 kepada 0.57. Nilai-nilai MAR ini mencadangkan bahawa semua pencilan ini berpunca

dari sumber yang berisiko tinggi. Kebanyakan pencilan adalah rintang terhadap penicillin (100%), ampicillin (100%), carbenicillin (95.24%),erythromycin (95.24%) dan bacitracin (71.43%). Sebahagian pencilan menunjukkan pelbagai tahap kerintangan terhadap cephalothin (28.57%), moxalactam (28.57%), kanamycin (19.05%), tetracycline (14.29%), asid nalidixic (9.52%), dan gentamicin (9.52%). Walau bagaimanapun, kesemua pencilan didapati sensitif terhadap streptomycin, norfloxacin, dan chloramphenicol. Tujuh pencilan didapati mempunyai satu plasmid, enam pencilan mempunyai dua plasmid, manakala lapan pencilan tidak mengandungi plasmid. Saiz plasmid adalah dari 2.6 MDa ke 35.8 MDa. Semua pencilan adalah pembawa gen *toxR* yang spesifik kepada *V. parahaemolyticus* tetapi semua pencilan tidak membawa gen *tdh*. Hanya satu pencilan membawa gen *trh*. Dua kaedah pencirian yang digunakan untuk mengkaji perbezaan serta persamaan genetik di antara pencilan yang dikumpul dari kawasan yang berlainan. Dalam analisis ERIC-PCR, primer ERIC 1R dan ERIC 2 telah digunakan. Melalui analisis ini, 15 profail ERIC yang berbeza dapat diperhatikan, menunjukkan kepelbagaian genetik yang luas di kalangan pencilan; pencilan dapat dibezakan kepada 2 sub-divisi dan 14 pencilan individu dengan berdasarkan kepada kesamaan pada 70% dendrogram yang dihasilkan. Dalam kaedah PFGE, enzim penghad *Sfi* I telah digunakan. Analisis terhadap profail PFGE telah membahagikan pencilan kepada 4 sub-divisi dan 9 pencilan individu berdasarkan kepada kesamaan 70% pada dendrogram yang dihasilkan. Walau bagaimanapun, kaedah ini mengalami degradasi DNA mengakibatkan 4

pencilan tidak dapat dicirikan. Kajian ini menunjukkan bahawa perairan laut Semenanjung Malaysia berisiko tinggi untuk *V. parahaemolyticus* patogenik yang boleh mencemarkan makanan laut yang ditangkap di kawasan kajian.

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The Lord is my shepherd; I have everything I need. ***Psalm*** **23**

I thank GOD the Almighty for His unlimited guide and giving me the wisdom and care unto me that I am able to finish this piece of work.

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To all my friends, church members and housemates- Thank you for your prayers and kindness, may God's blessing be upon all of you.

I certify that an Examination Committee met on 9th November 2004 to conduct the final examination of **Gwendelynne Bulan Tanil** on her **Master of Science** thesis entitled "**Molecular Characterization of *Vibrio parahaemolyticus* Isolated From The Coasts of Port Klang and Lukut located along Peninsular Malaysia"** in accordance with Universiti Pertanian Malaysia (Higher Degree) Act of 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommended that the candidate be awarded the relevant degree. The Committee Members for the candidate are as follows:

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

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DECLARATION

I hereby declare that the thesis is 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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GWENDELYNNE BULAN TANIL

Date: November 2004

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