UNIVERSITI PUTRA MALAYSIA

MOLECULAR CHARACTERIZATION, PLASMID PROFILING AND ANTIBIOTIC SENSITIVITY OF *VIBRIO PARAHAEMOLYTICUS* FROM SHELLFISH, HOSPITAL WASTEWATER AND HUMAN STOOLS SAMPLES IN PADANG, WEST SUMATERA, INDONESIA

MARLINA

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By

MARLINA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

May 2007

Dedicated to my loving husband, Ir. Adly Havendri, MSc and my children Aditya Rahmat, Adrian Faisal, Arief Darmawan and Putri Nadhira Adelina

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

MOLECULAR CHARACTERIZATION, PLASMID PROFILING AND ANTIBIOTIC SENSITIVITY OF *VIBRIO PARAHAEMOLYTICUS* FROM SHELLFISH, HOSPITAL WASTEWATER AND HUMAN STOOLS IN PADANG, WEST SUMATERA, INDONESIA

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May 2007

Chairman : Professor Son Radu, PhD

Faculty : Food Science and Technology

Vibrio parahaemolyticus, a gram-negative marine bacterium, is an important food borne pathogen causing gastroenteritis, particularly in areas with high seafood consumption.

A total of 29 human stools, 20 hospital wastewater and 120 shellfish samples from Padang area in Sumatera, Indonesia were examined in this study. Purple coloured colonies presumptive of *V. parahaemolyticus* from preliminary screening process on CHROMagar™ Vibrio were selected and confirmed as *V. parahaemolyticus* using polymerase chain reaction by the amplification of the *tox*R fragment at 368 bp. Of the 169 samples, 42 (24.8%) from shellfish (13 from *B. violacae*; 20 from *C. moltkiana*; 9 from *F. ater*), 13 (7.7%) from hospital wastewater and 12 (7.1%) from human stool samples were found to be contaminated with *V. parahaemolyticus*. The presence of

virulence genes (*tdh* and *trh*) of all *toxR* positive isolates were carried out using PCR. None of the isolates possesed the *trh* gene. However, 18 isolates from the human stools, hospital wastewater and shellfish (raw *B. violacae* and cooked *C. moltkiana*) samples harboured the *tdh* gene.

A total of 97 *V. parahaemolyticus* isolates from human stools, hospital wastewater and shellfish samples were examined for their resistance to 15 antibiotics. Majority of the isolates (70%) were resistant to more than nine antibiotics in this study. The general, a *V. parahaemolyticus* isolates are resistant to sulfamethoxazole (100%), rifampin (95%) and tetracycline (75%) and sensitive to norfloxacin (96%). None of the isolates from human stools were resistant to ampicillin. Overall, all isolates were sensitive to chloramphenicol and floroquinolones (ciprofloxacin and norfloxacin agents).

Eighty three isolates were examined for the existence of plasmids. A total of 61 (74.7%) *V. parahaemolyticus* isolates were plasmid-free. Nine isolates (11.8%) harboured the 9.4 kb plasmid. All of the remaining isolates carried plasmid DNA with sizes ranging from 2.3 kb to >23 kb. A large plasmid of 23 kb was evident in the plasmid harboring strains and appeared as the only plasmid in three isolates.

RAPD profiling with three primers (OPAR3, OPAR4 and OPAR8) produced four major clusters (R1, R2, R3 and R4) and 7 minor clusters (I, II, III, IV, V,

VI and VII). ERIC PCR using primer 1R and 2R produced two major clusters (E1 and E2) and ten minor clusters (A, B, C, D, E, F, G, H, I and J). All the dendrograms were being constructed utilizing the RAPDistance software package based on the data retrieved from the presence or absence of banding pattern. Both the molecular techniques of RAPD and ERIC genotyping showed most strains (? % and ?% respectively) from different samples and different locations revealed very high genetic variability in the microbial population studied. Combining the results of RAPD with ERIC apparently provides a degree of discrimination that should be adequate for identifying possible origins of *V. parahaemolyticus* contamination and for establishing relationships between isolates. Both methods showed a great diversity among the isolates of this species and could represent useful tools for the epidemiological typing of *V. parahaemolyticus* from Indonesia.

Hence, the problem of the contamination of foods by *V. parahaemolyticus*, like many other food safety issues in diverse society, reflects the challenges of larger public health systems of care. Addressing this problem will require a combined approach including improved access, legislation, education, and culturally relevant patient-provider interactions.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN SECARA MOLEKUL, PROFIL PLASMID DAN SENSITIVITI ANTIBIOTIK *VIBRIO PARAHAEMOLYTICUS* DARIPADA KERANG, AIR KUMBAHAN HOSPITAL DAN FECAL MANUSIA DARI KAWASAN PADANG, SUMATERA BARAT, INDONESIA

Oleh

MARLINA

May 2007

Pengerusi : Profesor Son Radu, PhD

Fakulti : Sains dan Teknologi Makanan

Vibrio parahaemolyticus, suatu bakteria marin gram negatif, adalah penyebab gastroenteritis bawaan makanan sedunia, terutamanya di kawasan yang pengambilan makanan lautnya adalah tinggi, dan merupakan patogen bawaan makanan yang penting di kebanyakan negara Asia.

Sejumlah 29 sampel "fecal" manusia, 20 sampel kumbahan hospital, dan 120 sampel kerang dari kawasan Padang di Sumatera, Indonesia dikaji. Beberapa teknik molekular dijalankan ke atas koloni berwarna ungu yang dipilih dari CHROMagar™ Vibrio yang diandaikan sebagai *V. parahaemolyticus* dengan menggunakan teknik reaksi rantai polymerase (PCR) mensasari gen *tox*R yang menjangkakan fragmen *tox*R 368bp. Kehadiran gen virulen (*tdh* dan *trh*) ke atas semua pencilan positif toxR dijalankan menggunakan PCR. Tiada pencilan yang menghasilkan band

yang dijangkakan untuk gen *trh*. Walau bagaimanapun, 18 pencilan daripada sampel "fecal" manusia, kumbahan hospital dan kerang (*B. violacae* mentah dan *C. moltkiana* yang dimasak) menghasilkan band 250bp yang dijangkakan daripada amplifikasi gen *tdh*. Keputusan daripada kajian ini menunjukkan bahawa *V. parahaemolyticus* telah dikesan di dalam pencilan daripada kerang (*B. violacae*, *C. moltkiana* dan *F. ater*), kumbahan hospital dan "fecal" manusia.

Sembilan puluh tujuh pencilan *V. parahaemolyticus* tersebut diuji untuk kesesuaiannya terhadap 15 antibiotik. 12 kluster sejajar dengan 97 pencilan (C1 hingga C12) dapat ditakrifkan dalam dendrogram dengan tahap kesamaan daripada 30% hingga 98% menggunakan perisian Bionumerics versi 4.6. Kesemua pencilan tersebut didapati menunjukkan kerintangan terhadap sekurang-kurangnya empat jenis antibiotik. Majoriti pencilan (70%) mempunyai kerintangan terhadap sembilan jenis antibiotik. Keseluruhannya, pencilan-pencilan *V. parahaemolyticus* berkelakuan rintang terhadap sulphamethoxazole (100%), rifampin (95%), dan tetracycline (75%) dan sensitif kepada norfloxacin (96%). Tiada pencilan daripada "fecal" manusia yang menunjukkan kerintangan terhadap ampicilin. *V. parahaemolyticus* yang dipencilkan daripada "fecal" manusia masih sensitif kepada ampicillin. Keseluruhannya, semua pencilan sensitif kepada chloramphenicol dan floroquinolones (agen ciprofloxacin dan norfloxacin). Sejumlah 61 (74.7%) daripada 83 pencilan *V. parahaemolitycus* yang diuji adalah bebas plasmid. 9 pencilan (11.84%) mempunyai 94 kb. Semua

pencilan yang lain membawa DNA plasmid daripada 2.3 kb hingga >23 kb. Plasmid besar 23 kb adalah nyata pada 3/22 pencilan yang mengandungi plasmid dan muncul sebagai satu-satunya plasmid dalam 3 pencilan.

Amplifikasi polimorfik DNA rawak (RAPD) PCR menggunakan tiga primer (OPAR3, OPAR4 dan OPAR8) menghasilkan empat kluster besar (R1, R2, R3 dan R4), 7 kluster kecil (I, II, III, IV, V, VI dan VII). Konsensus intergenik repetitif enterobakterial (ERIC) PCR menggunakan primer 1R dan 2R menghasilkan dua kluster besar (E1 dan E2) dan sepuluh kluster kecil (A, B, C, D, E, F, G, H, I dan J). Semua dendrogram dihasilkan menggunakan pakej RAPDistance berdasarkan data yang diperolehi daripada kehadiran atau ketidakhadiran paten band. Kedua-dua teknik molekular genotaiping RAPD dan ERIC menunjukkan beberapa pencilan daripada sampel dan lokasi berbeza dan menunjukkan variasi genetik yang sangat tinggi dalam populasi mikrobial yang dikaji. Menggabungkan keputusan RAPD dan ERIC dengan jelas memberikan darjah diskriminasi yang mencukupi untuk mengenalpasti sumber yang mungkin bagi kontaminasi *V. parahaemolyticus* dan untuk menetapkan hubungan antara pencilan. Kedua-dua kaedah menunjukkan kepelbagaian yang tinggi antara pencilan spesis ini dan dapat dijadikan alat berguna untuk taiping epidemiologikal *V. parahaemolyticus* di Indonesia.

Jadi, masalah kontaminasi makanan oleh *V. parahaemolyticus*, seperti kebanyakan isu keselamatan makanan dalam masyarakat pelbagai,

menggambarkan cabaran sistem penjagaan kesihatan awam yang lebih besar. Penyelesaian maslah ini memerlukan pendekatan kombinasi antara akses yang dikembangkan, legislasi, pendidikan dan interaksi pesakit-pembekal yang relevan secara budaya.

ACKNOWLEDGEMENTS

In the name of Allah, The Most Gracious and The Most Merciful First and foremost, I would like to express my heartfelt thanks to Almighty for giving me the will, strength and the perseverance to pursue and complete my PhD.

I would like to express my sincere gratitude and appreciation to the chairman of my supervisory committee, Professor Dr. Son Radu, for his invaluable guidance, dedicated efforts, supervision and continuous support throughout the study.

Sincere thanks and appreciation are also go to my co-supervisory, Associate Professor Dr. Suhaimi Napis, Dr. Sahilah Abdul Mutalib and Dr. Zunita Zakaria for their advices, suggestions and supports.

Sincere gratitude is also extended to all the staff of the Faculty of Food Science and Technology, Universiti Putra Malaysia, who contributed one way or another towards the completation of my study.

Also, I would like to express and extended my thank to Profesor. Dr. Mitsuaki Nishibuchi from Center for Southeast, Asian Studies, Kyoto University, Japan for his support and contribution.

My special thanks also to all my students in Department of Pharmacy, University of Andalas, Indonesia especially Haura, Budi, Sanil, Ndaru, Reti,

Yerli, Winda, Yuni, Amel, Indah, Kakang, Ayip, Dona, Rini, Dayu, Ijul, Ica, Sari, Ella, Imung and Eko, for assistance in sampling.

I am indebted to my dear friend, Cheah Yoke Kqueen for his willingness and constant assistance and help in time when I was having difficulties with my molecular works and help me to write scientific papers and poster. Words cannot express my gratitude for his supports.

I am grateful to my friends and my housemate, Lesley Maurice and Chandrika for their help, encouragement and for paying attention for all my problems. I am also grateful to my best friends Aida and her family, for her help, support and most of all a family to me during my study here. I love you.

I would like to thank all my friends in the Laboratory of Food Safety and Molecular Typing, Faculty of Food Science and Technology, UPM: Mas, Kqueen, Lesley, Chandrika, Yousr, Tung, Gwen, Belinda, Tuan Zainazor, Zila, Syila, Zul, Daniel, Rani, Sia Yen, Tosiah, Patrick, Azura, Henie and Jurin and Chai.

Lastly, heartfelt appreciation to be my loved family, my husband, Abang Adly, my children Adit, Yayan, Arief and Dhira, my mother Ibunda Hajjah

Syarifah and mother in law mama Hajjah Mahyar for their love and support, encouragement who share much of my joy and sorrow.

MARLINA

I certify that an Examination Committee has met on _____to conduct the final examination of Marlina on her Doctor of Philosophy thesis entitled "Isolation and Molecular Characterization of *Vibrio parahaemolyticus* from Shellfish, Environmental and Clinical Samples in Padang, West Sumatera Indonesia" 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 Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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Date: 13 September 2007

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.

_____

MARLINA

Date: 1 August

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LIST OF ABBREVIATIONS

APW	Alkaline Peptone Water
bp	base pair
CDC	Center of Disease Control and Prevention
CFU	Colony-Forming Unit
CT	Cholera Toxin
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanine triphosphate
DNA	Deoxyribo Nucleic Acid
dNTP	Deoxynucleotide triphosphate
dTTP	Deoxythymidine triphosphate
EDTA	Ethylene Diamine Tetra Acetic
ERIC	Enterobacterial Repetitive Intergenic Consensus
GET	Glucose-EDTA-Tris base
KAc	Kalium Acetate
kb	kilobase
KP	Kanagawa Phenomenon
LB	Luria Bertani
MDR	Multiple Drug Resistance
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
min	minute(m)
NaCl	Sodium chloride