UNIVERSITI PUTRA MALAYSIA

DETECTION OF VIBRIO CHOLERAE AND VIBRIO PARAHAEMOLYTICUS IN SEAFOOD USING MPN-PCR TECHNIQUE, AND THEIR MOLECULAR CHARACTERISTICS

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VENGADESH S/O LETCHUMANAN

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

DETECTION OF *VIBRIO CHOLERAE* AND *VIBRIO PARAHAEMOLYTICUS* IN SEAFOOD USING MPN-PCR BASED TECHNIQUE, AND THEIR MOLECULAR CHARACTERISTICS

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Chair: Associate Professor Cheah Yoke Kqueen, PhD

Faculty: Medicine and Health Sciences

Seafood is professed by consumers worldwide to be healthy and nutritious food due to abundance of scientific and documented health benefits. Approximately 90% of global aquaculture production is based in Asia. Nevertheless, recent food borne outbreaks are closely associated with seafood consumption. Seafood is known as a vehicle of transmission of food borne bacteria and causes human illness worldwide. In Malaysia, statistics in year 2009 have shown among the food and water borne diseases, food poisoning has the highest incidence rate of 36.17 per 100,000 populations and with a mortality rate of 0.01 per 100,000 populations. The purpose of this study is to apply and enumerate *Vibrio cholerae* and *Vibrio parahaemolyticus* from seafood samples by utilizing the Most Probable Number Method (MPN) and several molecular typing methods including polymerase chain reaction (PCR), enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) and plasmid profiling.

The application of conventional method using the Most Probable Number (MPN) method with selective enrichment broth and agar medium is very useful in isolating *Vibrio cholerae* and *Vibrio parahaemolyticus*. This method is coupled with PCR based method to obtain specific, sensitive and precise results. The densities enumerated by the MPN-Real Time PCR targeting *epsM* gene and the MPN-PCR targeting *toxR* gene is higher than those by MPN-Plate.

Genomic DNA of 104 *Vibrio cholerae* isolates was confirmed by a specific optimized multiplex PCR program targeting *hem* gene, *hlyA* gene, *ctx* gene and *zot* gene. 10 isolates were tested positive for cholera toxin gene (*ctx*) gene. All the isolates were positive to *hem* gene at 519bp and *hlyA* gene at 738bp but none was tested positive for *zot* gene. On the other hand, all the 100 *Vibrio parahaemolyticus* isolates were tested positive for regulatory gene, *toxR* yielded 368bp. The PCR amplification of the respective genes is a rapid and reliable method of detecting *Vibrio cholerae* and *Vibrio parahaemolyticus* isolates from seafood samples.

ERIC sequences are short, highly conserved 126 bp non-coding regions found in the *Enterobacteriaceae*. Its location in bacterial genomes allows discrimination at the genus, species and serovars levels. Dendrogram of ERIC-PCR were analyzed using the Bionumerics Version 6.0 (Applied Maths, Germany) software. From 104 isolates of *Vibrio cholerae*, ERIC-PCR with primers *ERIC-1* and *ERIC-2* produced 15 clusters and 9 single isolates at 40% similarity. Where else, 100 isolates of *Vibrio parahaemolyticus* produced 15 clusters and 6 single isolates at 40% similarity. The

results demonstrated that ERIC-PCR is a excellent tool for differentiation and characterization of *Vibrio* species.

Plasmids of *Vibrio cholerae* and *Vibrio parahaemolyticus* vary in size from 2.2 Kb to more than 7.4 Kb. Despite limited knowledge on their function, their presence is frequently used for strain differentiation in epidemiological studies. Plasmid profiling of 104 *Vibrio cholerae* isolates clustered into 19 groups based on the number and pattern of the bands. Where else, plasmid profiling of 100 *Vibrio parahaemolyticus* isolates clustered into 11 groups based on the number and pattern of the bands.

As a conclusion, the concern about possible health illness from *Vibrio* species, especially when seafood remains as a vehicle of transmission of *Vibrio*, will continue into the likely future. Therefore, to establish effective control measures to reduce the risk of this bacterium infection and to ensure the safety of foods, surveillance and epidemiology, the employment of molecular methods for the detection of *Vibrio cholerae* and *Vibrio parahaemolyticus* in food and environment is important. The results from this study could serve as vital information in *Vibrio* spp. epidemiology, surveillance, better infection control measures and support of public health policy.

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

PENGESANAN *VIBRIO CHOLERAE* AND *VIBRIO PARAHAEMOLYTICUS* DALAM MAKANAN LAUT DENGAN MENGGUNAKAN TEKNIK MPN-PCR, DAN MOLEKUL PENCIRIAN

Oleh

VENGADESH A/L LETCHUMANAN

July 2013

Pengerusi: Associate Professor Cheah Yoke Kqueen, PhD

Fakulti: Perubatan dan Sains Kesihatan

Makanan laut dikatakan oleh pengguna di seluruh dunia sebagai makanan yang sihat dan berkhasiat kerana mengandungi banyak manfaat kesihatan yang saintifik dan didokumenkan. Kira-kira 90% daripada global pengeluaran akuakultur berpusat di Asia. Walau bagaimanapun, baru-baru ini penyakit keracunan makanan berkait rapat dengan pengambilan makanan laut. Di Malaysia, statistik pada tahun 2009 telah menunjukkan antara keracunan makanan mempunyai kadar insiden tertinggi 36.17 setiap 100,000 penduduk dan dengan kadar kematian 0.01 setiap 100,000 populasi. Tujuan kajian ini adalah untuk mencirikan dan menghitung *Vibrio cholerae* dan *Vibrio parahaemolyticus* dalam sampel makanan laut dengan menggunakan kaedah 'Most Probable Number (MPN)', dan beberapa kaedah molekul yang termasuk 'polymerase chain reaction (PCR)', 'enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR)' dan profil plasmid.

Penggunaan kaedah konvensional menggunakan kaedah 'Most Probable Number (MPN)' dengan selektif agar media adalah sangat effektif dalam mengasingkan *Vibrio cholerae* dan *Vibrio parahaemolyticus*. Kaedah ini ditambah pula dengan kaedah berasaskan PCR bagi mendapatkan keputusan, sensitif dan tepat. Kepadatan disenaraikan oleh MPN-Real Time PCR mensasarkan gen *epsM* dan MPN-PCR mensasarkan gen *toxR* adalah lebih tinggi daripada MPN-Plate.

DNA genomik 104 *Vibrio cholerae* pencilan telah disahkan oleh suatu multipleks yang telah dioptimasi mensasarkan gen tertentu mengepung, gen *hlyA*, gen *hem*, gen *ctx* dan gen *zot*. 10 pencilan telah diuji positif untuk 'cholera toxin (*ctx*)'gen. Semua pencilan adalah positif untuk gen *hem* mengepung di 519bp dan gen *hlyA* di 738bp tetapi tiada sebarang pencilan yang menunjukkan keputusan positif apabila diuji dengan gen *zot*. Sebaliknya, semua 100 *Vibrio parahaemolyticus* pencilan telah diuji positif untuk gen 'regulatory', *toxR* menghasilkan 368bp. Amplifikasi PCR daripada gen masing-masing adalah kaedah yang pantas dan boleh dipercayai untuk mengesan *Vibrio cholerae* dan *Vibrio parahaemolyticus* pencilan daripada sampel makanan laut.

Urutan ERIC adalah pendek, sangat dipelihara 126 bp bukan kod dalam lingkungan dalam Enterobacteriaceae. Dendrogram daripada ERIC-PCR telah dianalisis menggunakan Version Bionumerics yang 6.0 (Gunaan Matematik, Jerman) perisian. Dari 104 pencilan *Vibrio cholerae*, ERIC-PCR dengan primers *ERIC-1* dan *ERIC-2* yang dihasilkan 15 kelompok dan 9 diasingkan tunggal pada 40% persamaan. Sementara, 100 pencilan *Vibrio parahaemolyticus* mengahasilkan 15 kelompok dan 6 diasingkan tunggal pada 40% persamaan. Keputusan ini menunjukkan bahawa

ERIC-PCR adalah alat yang sangat baik untuk pembezaan dan pencirian spesies *Vibrio*.

Profil plasmid *Vibrio cholerae* dan *Vibrio parahaemolyticus* dalam kajian ini berbeza dalam saiz daripada 2.2Kb kepada lebih daripada 7.4Kb. Walaupun pengetahuan yang terhad kepada fungsi mereka, kehadiran mereka sering digunakan untuk perbezaan tekanan dalam kajian epidemiologi. 104 *Vibrio cholerae* pencilan profil plasmid berkelompok ke dalam 19 kumpulan berdasarkan bilangan dan corak band. Manakala 100 *Vibrio parahaemolyticus* pencilan profil plasmid berkelompok kepada 11 kumpulan berdasarkan bilangan dan corak band.

Kesimpulannya, kebimbangan mengenai penyakit kesihatan yang mungkin daripada spesies *Vibrio*, terutamanya apabila makanan laut kekal sebagai kenderaan penghantaran *Vibrio* bakteria, akan berterusan pada masa depan yang mungkin. Oleh itu, untuk mewujudkan langkah kawalan yang berkesan bagi mengurangkan risiko jangkitan bakteria ini dan memastikan keselamatan makanan, pengawasan dan epidemiologi, pengambilan kaedah molekul untuk mengesan *Vibrio cholerae* dan *Vibrio parahaemolyticus* dalam makanan dan persekitaran adalah penting. Hasil daripada kajian ini boleh dijadikan sebagai maklumat penting dalam *Vibrio* spp. epidemiologi, pengawasan, langkah-langkah kawalan jangkitan yang lebih baik dan menyokong dasar kesihatan awam.

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I certify that a Thesis Examination Committee has met on 2nd July 2013 to conduct the final examination of Vengadesh a/l Letchumanan on his thesis entitled "Detection of *Vibrio cholerae* and *Vibrio parahaemolyticus* in Seafood Using MPN-PCR Based Technique, and Their Molecular Characteristics" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis was 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 were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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VENGADESH S/O LETCHUMANAN

Date: 2 July 2013

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