UNIVERSITI PUTRA MALAYSIA

ISOLATION AND MOLECULAR CHARACTERIZATION OF VIRULENT AND AVIRULENT STRAINS OF *VIBRIO ALGINOLYTICUS*

RASHIDAH BINTI ABDUL RAZAK

IB 2013 1

ISOLATION AND MOLECULAR CHARACTERIZATION OF VIRULENT AND AVIRULENT STRAINS OF *VIBRIO ALGINOLYTICUS*

By

RASHIDAH BINTI ABDUL RAZAK

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

January 2013

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

ISOLATION AND MOLECULAR CHARACTERIZATION OF VIRULENT AND AVIRULENT STRAINS OF *VIBRIO ALGINOLYTICUS*

By

RASHIDAH ABDUL RAZAK

January 2013

Chairperson: Professor Dato' Mohamed Shariff Mohamed Din, PhD

Faculty: Institute of Bioscience

Vibriosis caused by *Vibrio alginolyticus* has been recognised as serious disease problem. Although *V. alginolyticus* were reported to be pathogen but it also been used to formulate probiotics used in shrimp aquaculture. Therefore, indigenous marine strains of *V. alginolyticus* were isolated and selected to study their virulence in shrimp postlarvae. The bacteria were isolated from 20 water and 20 sediment samples collected from natural environment and shrimp ponds in Selangor and Sabah, Malaysia. From the total of 79 isolates which formed yellow colonies on TCBS agar plates, 15 were identified as *V. alginolyticus* using *gyr*B gene based PCR and showed 94 to 100% similarity to the partial sequence of *V. alginolyticus* 16S rRNA gene. From the 15 isolates, only six were chosen for pathogenicity study which consisted of one beta, three alpha and two gamma hemolysis type of strains. After 24 hours of incubation in TSB with shaking at 30 °C, these isolates showed different OD readings and the highest was 1.7554 ± 0.0031 with cell number of 4.72 X 10^9 ± 2.74 X 10^8 CFU·mL^{-1} indicating that *V. alginolyticus* M2 showed the best growth rate in TSB with 1.5% NaCl compared to other isolates.

In the pathogenicity test, three treatments consisting of different concentrations of bacteria (10^3, 10^5 and 10^7 CFU·mL^{-1}) and one control group contained physiological saline were performed on shrimp postlarvae. There was a significant difference ($p<0.05$) in the mortality rate of the postlarvae between the three treatments used and the tests revealed that the mortality rate of the shrimp postlarvae increased with duration of exposure. The highest mortality was noted in *V. alginolyticus* M13 tanks at concentration of 1.28 X 10^7 CFU·mL^{-1} where the average mortality was 98.4 ± 1.7%. Furthermore, the LD_{50} study showed that the lowest value was 4.33 X 10^4 CFU·mL^{-1} for *V. alginolyticus* M13 and the highest value was 8.11 X 10^9 CFU·mL^{-1} for *V. alginolyticus* M2 indicating the isolates varied in virulence. The isolates were characterized as highly virulent were *V. alginolyticus* M13 and S7, weakly virulent were *V. alginolyticus* G3 and S48 and avirulent were *V. alginolyticus* M2 and G34. In order to distinguish between virulent and avirulent strains, four primers were used in RAPD analysis. From this, we deduced that only one primer (GTG_5 primer) was suitable to characterise six strains of *V. alginolyticus*. Using the fingerprint patterns obtained, a dendrogram was constructed; these strains were divided into two main clusters, two subclusters and two different groups. The homogeneity between the strains ranged from 11.8 to 80.0%. The genetic distances between the virulent strains were 37.5% and 23.5% for avirulent strains which classified them into two distinct clusters and subclusters respectively. The weakly virulent strains showed high homogeneity as their genetic similarity was 62.5%. In addition, the strains isolated from the same source also showed genetic variation. For example, strains M2 and M13, S7 and S48 isolated from same shrimp farm showed only 41.7% and 53.3% homogeneity respectively. The results showed that isolates from the same source and having same virulence degree varied in their genetic profiles.

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

PEMENCILAN DAN PERINCIAN MOLEKULAR BAGI STRAIN AKUT DAN TIDAK AKUT *VIBRIO ALGINOLYTICUS*

Oleh

RASHIDAH ABDUL RAZAK

Januari 2013

Pengerusi: Professor Dato' Mohamed Shariff Mohamed Din, PhD

Fakulti: Institut Biosains

Vibriosis disebabkan oleh *Vibrio alginolyticus* telah dikenali sebagai masalah penyakit. Walaupun *V. alginolyticus* dilaporkan sebagai patogen tetapi ia juga diformulasikan sebagai probiotik dalam akuakultur udang. Oleh itu, *V. alginolyticus* strain tempatan dipilih untuk kajian keakutannya terhadap pascalarva udang. Bakteria ini diambil dari 20 sampel tanah dan 20 sampel air dari persekitaran semulajadi dan kolam udang di Selangor dan Sabah, Malaysia. Daripada 79 isolat membentuk koloni kuning di atas piring TCBS agar, 15 dikenalpasti sebagai *V. alginolyticus* menggunakan tindakbalas polimer berantai berasaskan gen *gyr*B dan menunjukkan 94 hingga 100% persamaan dengan sebahagian jujukan gen 16S rRNA *V. alginolyticus*. Daripada 15 isolat, enam dipilih untuk kajian patogenisiti dimana terdiri dari jenis hemolisis satu beta, tiga alfa dan dua gamma. Selepas pengeraman 24 jam dalam TSB dengan goncangan pada 30 °C, isolat ini menunjukkan perbezaan bacaan OD dan bacaan paling tinggi adalah 1.7554 ± 0.0031 dengan bilangan sel 4.72 X 10^9 ± 2.74 X 10^8 CFU·mL^{-1} menandakan *V. alginolyticus* M2 mempunyai kadar pertumbuhan terbaik dalam TSB dengan 1.5% NaCl berbanding isolat lain.

Dalam ujian patogenisiti, tiga rawatan terdiri daripada kepekatan bakteria berbeza (10^3, 10^5 dan 10^7 CFU·mL^{-1}) dan satu kumpulan kawalan mengandungi fisiologikal saline dijalankan ke atas pascalarva udang. Terdapat perbezaan signifikan ($p<0.05$) bagi kadar kematian pascalarva diantara ketiga rawatan digunakan dan ujian menunjukkan kadar kematian bagi pascalarva meningkat dengan peningkatan masa pendedahan. Kematian tertinggi diperhatikan dalam tangki *V. alginolyticus* M13 pada kepekatan 1.28 X 10^7 CFU·mL^{-1} dimana purata kematian adalah 98.4 ± 1.7%. Tambahan pula, kajian LD_{50} menunjukkan nilai terendah adalah 4.33 X 10^4 CFU·mL^{-1} bagi *V. alginolyticus* M13 dan nilai tertinggi adalah 8.11 X 10^9 CFU·mL^{-1} bagi *V. alginolyticus* M2 menandakan isolat ini berbeza keakutannya. Isolat ini dipercirikan sebagai akut adalah *V.alginolyticus* M13 dan S7, akut rendah adalah G3 dan S48 dan tidak akut adalah M2 dan G34. Bagi membezakan strain akut dan tidak akut, empat primer digunakan dalam analisis amplifikasi polimorfik DNA rawak. Daripada ini, kami mengurangkan kepada satu primer (GTG_5 primer) sesuai untuk mempercirikan enam strain *V. alginolyticus*. Dengan menggunakan corak cap jari dimana satu dendrogram dibina; strain ini dibahagikan kepada dua kluster utama, dua subkluster dan dua kumpulan berbeza. Kehomogenan diantara semua isolat adalah 11.8 hingga 80.0%. Jarak genetik diantara strain akut adalah 37.5% dan 23.5% bagi strain tidak akut dimana mereka dikelaskan dalam kluster dan subkluster berbeza. Strain sederhana akut menunjukkan kehomogenan tinggi dimana persamaan genetiknya adalah 62.5%. Tambahan, strain diambil dari sumber sama juga menunjukkan variasi genetik. Sebagai contoh, strain M2 dan M13, S7 dan S48 mempunyai kehomogenan 41.7% dan 53.3% walaupun diambil dari kolam udang sama. Oleh itu, keputusan ini menunjukkan bahawa variasi genetik boleh berlaku walau dalam strain *V. alginolyticus* mempunyai persamaan tahap keakutan dan tempat sumber diambil.

ACKNOWLEDGEMENTS

I would like to acknowledge my main supervisor, Prof. Dato' Dr. Mohamed Shariff Mohamed Din, Faculty of Veterinary Medicine, Universiti Putra Malaysia for his advice and guidance. He inspired me greatly throughout the research and transcription of this thesis. His willingness to encouragement and motivate me have provided a good basis for the present thesis. His patience, enthusiasm and immense knowledge have supporting me during my difficult moments. His concern and financial assistance has contributed great value for me. I could not have imagined having a better advisor and mentor for my study.

My sincere gratitude to Prof. Dr. Fatimah Md. Yusoff, Marine Science and Aquaculture Laboratory, Institute of Bioscience, Universiti Putra Malaysia, member of my supervisory committee for her encouragement, constructive comments and guidance throughout the study. Her concern and willingness to help has been great support to me. I am grateful to Prof. Dr. Mariana Nor Shamsudin, Marine Science and Aquaculture Laboratory, Institute of Bioscience, Universiti Putra Malaysia, member of my supervisory committee for her kind suggestion and supervision throughout this study.

My heartily thankful to Dr. Lee Kok Leong and Dr. Sanjoy Banerjee, who helped me to have better understanding of this study. Their valuable advice and friendly help have been helpful for my study. I thank my fellow labmates for the encouraging discussion, for the overnight we were working together in laboratory and for all fun we have had in this few years. I also want to thanks all the laboratory staffs that helped me providing the materials and instruments for my research.

Last but not the least, I thanked my family: my parents Abdul Razak and Samsiah, for giving me moral and loving support throughout my life. My special gratitude to my brother and sisters for their loving supports. I owe my loving thanks to my husband, Muhammad Farizal Abdul Halim and my daughter, Malikha Aleesya for their understanding and constantly keeping me cheer up.

I certify that an Examination Committee has met on date of viva voce to conduct the final examination of **Rashidah binti Abdul Razak** on her degree thesis entitled "**Isolation and Characterisation of Virulent and Avirulent Strains of *Vibrio alginolyticus***" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were as follows:

Dato' Abdul Rani Bahaman, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malysia
(Chairman)

Siti Khairani Bejo, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malysia

Mohd Zamri Saad, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malysia

Kuo-Kau Lee, PhD
Professor
Department of Aquaculture
National Taiwan Ocean University
Taiwan
(External Examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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:

Mohamed Shariff Mohamed Din, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malysia
(Chairman)

Fatimah Md. Yusoff, PhD
Professor
Faculty of Science
Universiti Putra Malysia
(Member)

Mariana Nor Shamsudin, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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.

RASHIDAH ABDUL RAZAK

Date: 4 January 2013

TABLE OF CONTENTS