UNIVERSITI PUTRA MALAYSIA

INDIGENOUS IMMOBILIZED NITRIFYING BACTERIA FOR REDUCTION OF AMMONIA IN SHRIMP LARVICULTURE SYSTEM

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IB 2012 19

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By

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

November 2012

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

INDIGENOUS IMMOBILIZED NITRIFYING BACTERIA FOR REDUCTION OF AMMONIA IN SHRIMP LARVICULTURE SYSTEM

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Chairperson: Prof. Dato' Mohamed Shariff Mohamed Din, PhD

Faculty/Institute: Institute of Bioscience

The shrimp industry has grown rapidly due to the increase in market demand, which in turn resulted in intensive shrimp postlarvae (PL) cultivation practices. The ultrahigh stocking densities and over loading of feeds cause serious water quality problems in PL hatchery system. Exposure of shrimp PLs to toxic compounds like ammonia leads to stress and diseases that eventually causes PL mortality. The present study investigated the ability of local indigenous immobilized nitrifying bacteria, isolated from the marine environment, to reduce total ammonia nitrogen (TAN) in a shrimp larviculture system. For isolation of nitrifying bacteria, soils and water samples were collected from the west coast of Peninsular Malaysia such as Kuala Juru, Kuala Gula, Morib and shrimp farms in Kuala Langat. Bacteria were grown in Skinner and Walker medium and then screened for their ability to reduce ammonia. Since nitrifying bacteria cannot be isolated in a pure culture condition, they were cultured and maintained as consortia. The nitrifying bacteria consortia were then tested for their ability to reduce ammonia and the consortium that showed the lowest ammonia reading was selected for further experiments. Consortium M1, isolated from the mangrove area of Morib, showed the best reduction rate (0.154 mg/l/day) of ammonia from 2.0 ± 0.1 mg/l to 0.06 ± 0.01 mg/l in 14 days, when tested in the laboratory. Using 16S rDNA primers, three species of bacteria were identified from the consortium M1, namely *Pseudomonas aeruginosa* with 99%

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homology, *Pseudomonas stutzeri* with 98% homology and *Nocardioides albus* with 98% homology after BLAST analyses in Genebank. Further experiments were conducted to test the efficacy of the consortium M1 for its ability to reduce ammonia in a shrimp larviculture system. The bacteria consortium M1 was immobilized using alginate solution. Tanks treated with immobilized bacteria were able to achieve high ammonia reduction proficiency to 76.52%, followed by tanks that were replaced with 50% fresh seawater on alternate days (68.10 %), tanks treated with non-immobilized bacteria (36.84%) and tanks treated with alginate beads without bacteria (18.37%). However, in control tanks (without treatment) ammonia increased to 40.79% at 14 days. Tanks treated with immobilized bacteria has significantly ($P < 0.05$) increased survival rate (72.44%) and specific growth rate (SGR) (12.86%) of shrimp PL compared to other treatments.

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

BAKTERIA NITRIFIKASI TEMPATAN YANG DIIMMOBILISASIKAN BAGI PENGURANGAN AMMONIA DARI SISTEM LARVIKULTUR UDANG

Oleh

MAYA ERNA BINTI NATNAN

November 2012

Pengerusi: Prof. Dato' Mohamed Shariff Mohamed Din, PhD

Fakulti/ Institut: Institut Biosains

Industri udang berkembang pantas menyebabkan kultur pascalarva (PL) udang menjadi intensif dan seterusnya peningkatan permintaan pasaran. Kepadatan stok yang tinggi dan makanan berlebihan boleh menyebabkan masalah kualiti air di pusat penetasan pascalarva. Pendedahan PL udang kepada sebatian toksik seperti ammonia menjadi punca tekanan dan penyakit yang seterusnya menyebabkan punca kematian PL. Kajian ini menyiasat kemampuan pencilan bakteria nitrifikasi tempatan dari persekitaran marin yang telah diimmobilisasi, dalam mengurangkan jumlah ammonia nitrogen (TAN) dari sistem larvikultur udang. Sampel tanah dan air dari persekitaran marin diambil di sepanjang pantai barat Semenanjung Malaysia seperti Kuala Juru, Kuala Gula, Morib dan ladang kolam udang di Banting, Kuala Langat. Bakteria dibiakkan dalam media Skinner dan Walker, kemudian disaring untuk mengenalpasti bakteria yang boleh mengurangkan ammonia. Disebabkan bakteria tidak dapat dipelihara dalam keadaan kultur tulen, ia dikultur dan dipelihara dalam keadaan perkongsian. Perkongsian bakteria nitrifikasi kemudian diuji untuk keupayaan mengurangkan ammonia, dan perkongsian yang menunjukkan bacaan ammonia paling rendah telah dipilih untuk kajian lanjutan. Kultur perkongsian M1 yang diambil dari kawasan bakau di Morib menunjukkan tahap pengurangan ammonia (0.154 mg/l /hari) yang paling baik iaitu dari 2.0 ± 0.01 mg/l kepada 0.06 ± 0.01 mg/l

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dalam masa 14 hari apabila diuji di makmal. Dengan menggunakan primer 16S rDNA, tiga spesies bakteria telah dikenalpasti dari perkongsian M1, iaitu *Pseudomonas aeruginosa* dengan homologi 99%, *Pseudomonas stutzeri* dengan homologi 98% dan *Nocardioides albus* dengan homologi 98% selepas analisis BLAST di Genbank. Eksperimen selanjutnya dilakukan bagi menguji keberkesanan perkongsian M1 dalam kebolehan mengurangkan ammonia di dalam sistem tangki larvikultur udang. Tangki yang dirawat dengan bakteria immobilisasi menunjukkan keberkesanan paling baik dalam pengurangan ammonia (76.52%), diikuti oleh tangki yang telah digantikan dengan 50% air laut selang sehari (68.84%), tangki yang dirawat dengan bakteria yang tidak diimmobilisasi (36.84%) dan tangki yang dirawat dengan manik alginat tanpa bakteria (18.37%). Bagaimanapun, air di dalam tangki - kawalan (tanpa rawatan), menunjukkan penambahan ammonia kepada 40.79% selepas 14 hari. Tangki yang dirawat dengan bakteria immobilisasi menunjukkan signifikasi ($P < 0.05$) lebih tinggi kadar hidup (72.44%) dan kadar pertumbuhan tentu (SGR) (12.86%) bagi PL udang jika dibandingkan dengan rawatan lain.

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ACKNOWLEGEMENT

Assalammualaikum. Praise to Allah (S.W.T) the all powerful, the most wise and the most merciful, and greetings to His Messenger, the Saviour of mankind, Prophet Muhammad (S.A.W), his family, Moslems present and past. To Allah the Almighty, belong all praise and glory. Without His will and blessing, I would not have succeeded in completing this thesis.

I would like to express my heartfelt thanks and gratitude to my supervisor Prof. Dato' Dr. Mohamed Shariff Mohamed Din, Faculty of Veterinary Medicine, UPM for his valuable guidance and supervision towards completing the course of research and this thesis. His meticulous nature in evaluated my thesis has made this thesis a valuable document. Not to forget Prof. Dr. Fatimah Md Yusoff, Faculty of Agriculture, UPM, member of my advisory committee for her guidance and encouragement throughout the course of study.

I am also thankful to Dr. Sanjoy Banerjee and Dr. Helena Khatoon, who have been very helpful in contributing the necessary information and needs during the course of my study. Last but not least, I wish to thank my beloved family, labmates and colleagues for their patience, opinion, motivation, support and understanding throughout the process in finishing this research course and preparing this thesis.

Dearest beloved, all the experiences we have been through together and your encouragement have inspired me to develop myself personally and professionally. My thanks to Universiti Putra Malaysia for giving me the opportunity for pursue my Master's degree.

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APPROVAL SHEET

I certify that an Examination Committee has met on 6th November 2012 to conduct the final examination of Maya Erna binti Natnan, on her Master thesis entitled "Indigenous Immobilized Nitrifying Bacteria for the Reduction of Ammonia in Shrimp Larviculture System" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Pertanian Malaysia [P.U. (A) 106 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the 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 fulfillment of the requirement for the degree of Master in Science. The members of the Supervisory Committee were as follows:

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DECLARATION

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

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