UNIVERSITI PUTRA MALAYSIA

BACTERIAL FLORA ASSOCIATED WITH HATCHERY-REARED JUVENILE GIANT TIGER PRAWN, *Penaeus monodon* (FABRICIUS), AND SCREENING OF PUTATIVE BACTERIA AS PROBIOTIC CANDIDATE

SHAHRAM SHAKIBA ZADEH

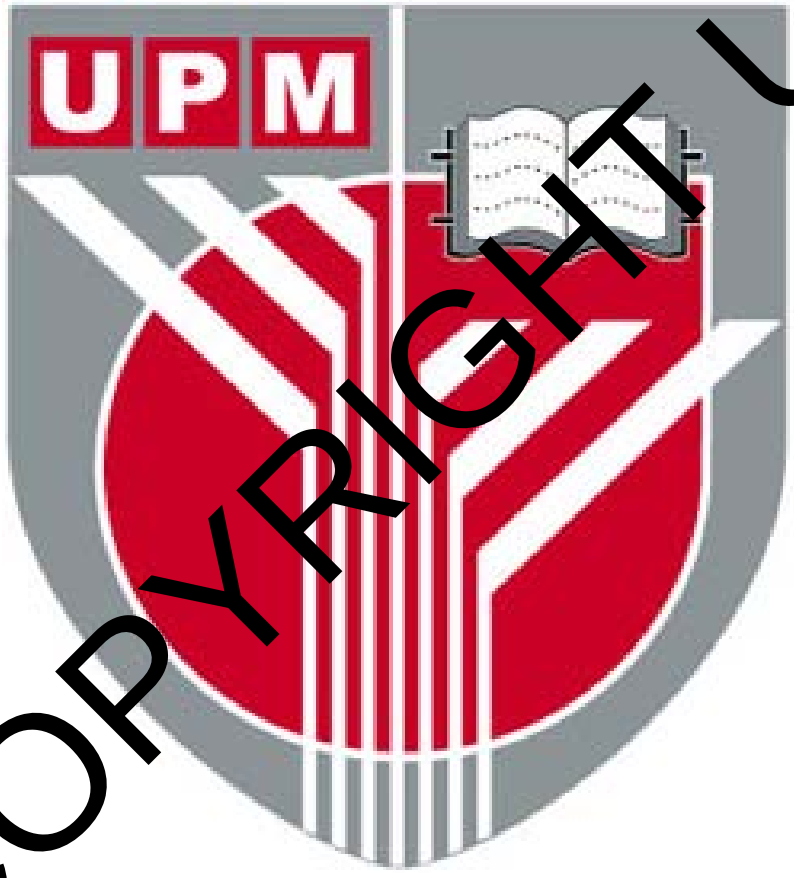
FP 2012 18

BACTERIAL FLORA ASSOCIATED WITH HATCHERY-REARED JUVENILE GIANT TIGER PRAWN, *Penaeus monodon* (FABRICIUS), AND SCREENING OF PUTATIVE BACTERIA AS PROBIOTIC CANDIDATE

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

February 2012

DEDICATION



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of philosophy

BACTERIAL FLORA ASSOCIATED WITH HATCHERY-REARED JUVENILE GIANT TIGER PRAWN, *Penaeus monodon* (FABRICIUS), AND SCREENING OF PUTATIVE BACTERIA AS PROBIOTIC CANDIDATE

By

SHAHRAM SHAKIBA ZADEH

Chairma

Faculty:

Post larva in Puchong hatchery ge. Bacterial flora of c determined using cor g microplate system. H nt and total *Pseudom* y digestive system (hepatopancreas and intestine). Seven different genera were identified from different body parts, sediment and rearing water comprised of 72% Gram negative bacteria. The predominant bacteria were *Vibrio* followed by *Shewanella*, while incident of *Shewanella* in digestive system was considerably more than sediment, which is indirect evidence for its colonization in digestive system. Thereafter, antagonistic ability of bacterial flora isolates was examined against the most detrimental shrimp pathogens

(*Vibrio harveyi*, *Vibrio parahaemolyticus,* and *Vibrio alginolyticus*) using well plate diffusion technique and cross streak technique. The largest inhibitory zones against mentioned shrimp pathogen were produced by *Shewanella algae* followed by *Burkholderia glumae* and *Bravibacterium linens*. Further antagonistic studies were conducted on *S. algae* in different pH, Salinity, temperature and time using Response Surface methodology. *S.algae* was able to induce its antagonistic ability against mentioned shrimp pathogens in relatively [illegible] eration on growth p[illegible] pH, salinity, temperatu[illegible] ate probiotic was abl[illegible] in different environm[illegible] e candidate probiotic [illegible] by means of a ranking [illegible] ubling time, d_t, can o[illegible] faster than *V. harvey*[illegible] olyticus*. The safety of [illegible] examined. Four tr[illegible] 2 levels of candidate [illegible] egative and positive controls for 3 weeks. The highest survival rate was observed in the 2nd treatment (10^{10} cfu/g) which was significantly different with controls. Low and high salinity stress tests and low temperature stress test were performed within treatment groups to evaluate the effect of candidate probiotic during stressful condition. The 2nd treatment (10^{10} cfu/g) exhibited significantly higher survival rate within treatments in all stress tests. Consequently, *in*

vivo study of candidate probiotic was assessed through feeding shrimp treatment groups by supplementing commercial shrimp feed with 4 levels of candidate probiotic (T_1; 10^4, T_2; 10^7, T_3; 10^{10} and T_4; 10^{13} cfu/g). The significant highest feed conversion ratio, specific growth rate, protein efficiency ratio and survival was observed in 3rd treatment, this treatment consisted of significantly better bacterial balance and exhibited highest survival rate after 2 weeks challenging with *V. harveyi*. The possible human risk asso dose 50%, LD_{50}, on robiotic was about 10 s safety for mammali aracteristics and can b s.

Abstrak Tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat untuk mendapatkan ijazah Doktor Falasafah

FLORA BAKTERIA TERLIBAT DALAM PELIHARAAN JUVENIL UDANG HARIMAU, *Penaeus monodon* (FABRICIUS), DARI HACERI DAN PENAPISAN BAKTERIA SEBAGAI CALON PROBIOTIK

Oleh

SHAHRAM SHAKIBA ZADEH

Pengeru

Fakulti: F

Pasca la a di Stesen

Penyelidi a sehingga

peringkat gian badan,

sedimen enggunakan

kaedah b mikroplate.

Jumlah mlah kiraan

Pseudom am sistem

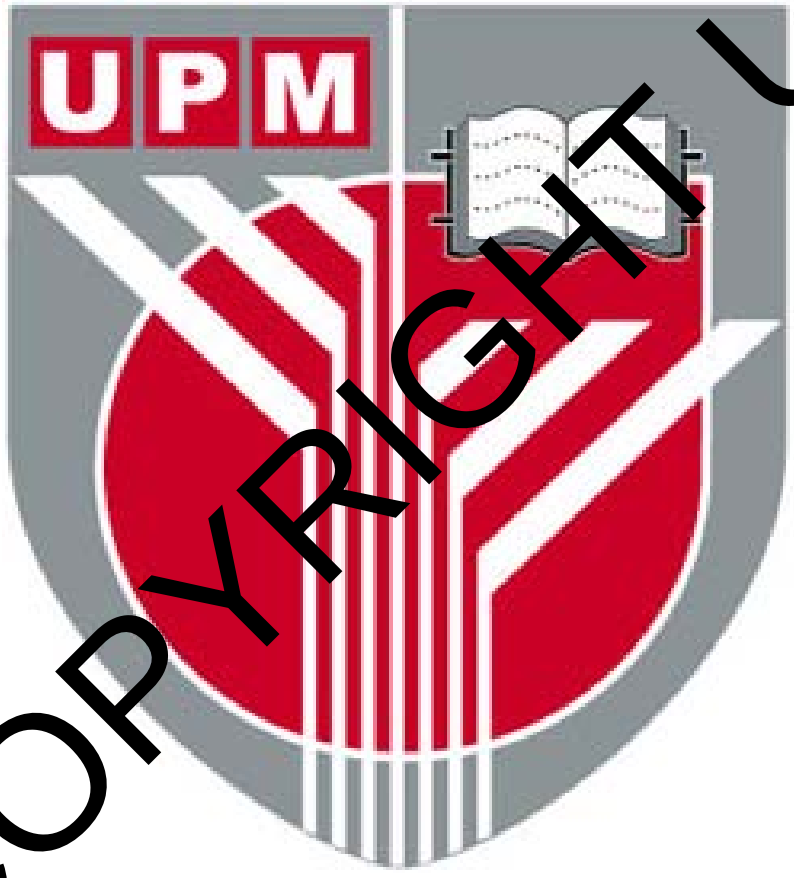
pencernaan (hepatopancrease dan usus). Tujuh genus yang berbeza telah dikenal pasti dari beberapa bahagian badan, sedimen dan air yang merupakan 72% bakteria Gram negatif. Bakteria utama ialah *Vibrio* diikuti oleh *Shewanella*, manakala kehadiran *Shewanella* dalam sistem pencernaan adalah lebih banyak dari dalam sedimen, menunjukkan bukti kolonisasi dalam sistem pencernaan. Seterusnya, keupayaan antagonis flora bakteria

yang diasingkan dilakukan terhadap patogen udang yang paling merbahaya (*Vibrio harveyi*, *Vibrio parahaemolyticus,* and *Vibrio alginolyticus*) dengan menggunakan teknik resapan piring dan teknik coretan silang. Zon penghalang yang paling terbesar terhadap patogen udang tersebut telah dihasilkan oleh *Shewanella algae* dikikuti oleh *Burkholderia glumae* dan *Bravibacterium linens.* Kajian lanjutan kemampuan antagonis telah dilakukan terhadap *S. algae* didalam pH, saliniti, suhu dan m yang berbeza n. *S. algae* mampu ja patogen udang te aan kajian. Pemerha robiotik, *S. Algae* da h dilakukan dengan k engeluarkan bahan a g berbeza. Seterusn ibandingkan dengan 3 IR indeks. Bakteria an tempoh bergan a lain. Calon p obiotik *gionolyticus* tapi tidak lebih cepat daripada *V. parahaemolyticus*. Keselamatan calon probiotik untuk udang harimau telah kaji berikutnya, dimana 4 rawatan kumpulan juvenil *P. monodon* diberi makan mengandungi calon probiotik dalam 2 peringkat (10^7 dan 10^{10} cfu / g), kawalan negatif dan positif selama 3 minggu. Kadar kemandirian yang tertinggi telah diperhatikan dalam rawatan 2 (10^{10} cfu / g) yang ketara berbeza dengan kawalan. Ujian tekanan rendah

dan tinggi bagi kemasinan dan suhu telah dijalankan dalam kumpulan rawatan untuk menilai kesan calon probiotik semasa keadaan tertekan. Rawatan 2 (10^{10} cfu / g) mempamerkan daya hidup yang lebih tinggi dengan ketara dalam tempoh rawatan dalam semua ujian tekanan. Sehubungan itu, dalam kajian *in vivo* calon probiotik telah dinilai melalui pemberian makanan tambahan komersil mengandungi 4 peringkat calon probiotik T1: 10^4 T2: 10^7, T3; 10^{10} dan T4; 10^{13} cfu / g). Nilai ketara tertinggi nisbah penukaran, kadar pe kemandirian diperhatik seimbangan bakteria ng tertinggi selepas risiko yang mungkin an *S. algae* telah dipe amalia, tikus BALB / pemberian melalui m alia. Secara umumny up baik dan boleh di omersil dan perindust

ACKNOWLEDGEMENTS

My full praise to the God for providing me with helpful supervisory team, cooperative real friends, supportive family and a heart filled with hope, which was enabled me to complete my study during toughest period of my life.



I certify that an Examination Committee met on 22nd February 2012 to conduct the final examination of Shahram Shakiba Zadeh on his Doctor of Philosophy thesis entitled Bacterial flora Associated with Hatchery-Reared Juvenile Giant Tiger Prawn, *Penaeus monodon* (fabricius), and Screening of Putative Bacteria as probiotic Candidate 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 candidate be awarded the Doctor of Philosophy.

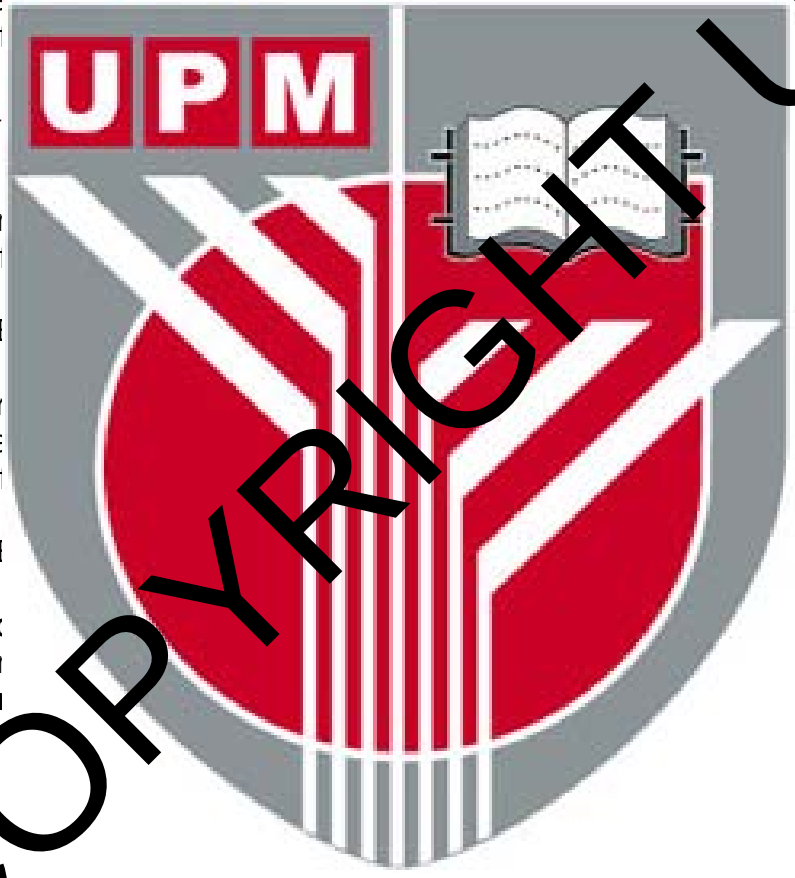
Members of the Examination Committee are as follows:

Mihdzar Bin Abdul Kadir, PhD
Associate Professor
Faculty of
Universiti
(Chairma

Aziz Bin
Professor
Faculty of
Universiti
(Internal E

Muta Har
Associate
Faculty of
Universiti
(Internal E

Mehdi Do
Professor
Primary In
South Au
Level 1,
(Externa



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

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Che Roos Saad, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Mohd Sal
Associate
Faculty o
Universiti
(Member)

Annie Ch
Lecturer
Faculty o
Universiti
(Member)

Kamaruz
Associate
Faculty o
Universiti
(Member)

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

Date:

DECLARATION

I declare that this 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, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

_____

SHAHRAM SHAKIBA ZADEH

2012

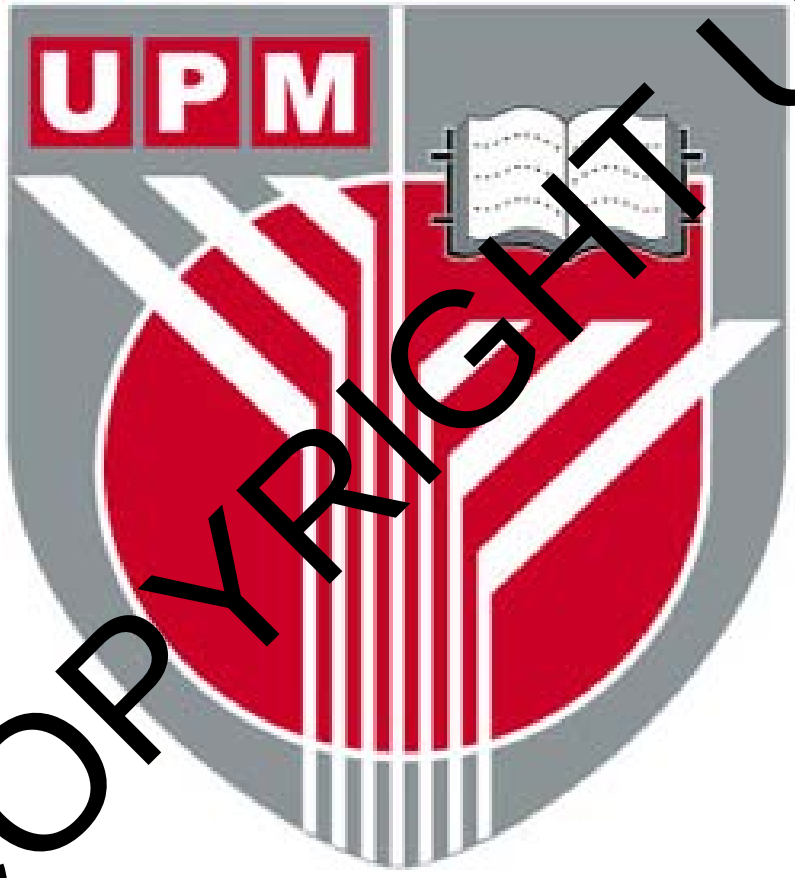


TABLE OF CONTANTS