



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

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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



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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



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72% Gram negative bacteria. The predominant bacteria were *Vibrio* followed

by *Shewanella*, while incident of *Shewanella* in digestive system was

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(*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 low salinity. The effect of *S. algae* on growth of *V. parahaemolyticus* was studied in different pH, salinity, temperature and time. *S. algae* was able to induce its antagonistic ability against mentioned shrimp pathogens in different environments. The effect of *S. algae* on growth of *V. parahaemolyticus* was studied in different environments. The candidate probiotic was evaluated by means of growth curve, doubling time, lag phase, and D_{90} ; faster than *V. parahaemolyticus*. The safety of *S. algae* was examined. Four treatments were used: 1st treatment (10¹⁰ cfu/g), 2 levels of *S. algae* (10¹⁰ cfu/g and 10¹¹ cfu/g), and positive controls for 3 weeks. The highest survival rate was observed in the 2nd treatment (10¹⁰ 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 (10¹⁰ 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 (T₁; 10⁴, T₂; 10⁷, T₃; 10¹⁰ and T₄; 10¹³ 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 associated with the probiotic was assessed by determining the LD₅₀, on about 10 mammalian and can be used as a safety for characteristics.



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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 SHAKIRA ZADEH



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Pseudom

perubahan (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

a di Stesen

a sehingga

jian badan,

gunakan

mikroplate.

umlah kiraan

am sistem



yang diasingkan dilakukan terhadap patogen udang yang paling berbahaya (*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 media yang berbeza. *S. algae* mampu menghalang pertumbuhan patogen udang terhadap kajian. Pemerhatian probiotik, *S. Algae* dan dengan k... h dilakukan dengan k... mengeluarkan bahan al... ig berbeza. Seterusnya... ibandingkan dengan 3... IR indeks. Bakteria... lan tempoh bergan... lain. Calon probiotik... *lgionolyticus* 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 peranakan, kadar pelepasan, dan ketahanan terhadap tekanan ketahanan, diperhatikannya keseimbangan bakteria, dan ketahanan terhadap tekanan ketahanan tertinggi selepas : risiko yang mungkin an *S. algae* telah dipelajari, tikus BALB / C, pemberian melalui n, alia. Secara umumnya, up baik dan boleh dipasarkan komersil dan perindustri.



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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.



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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.

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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.


SHAHDAM SHAHRA ZADEH

2012



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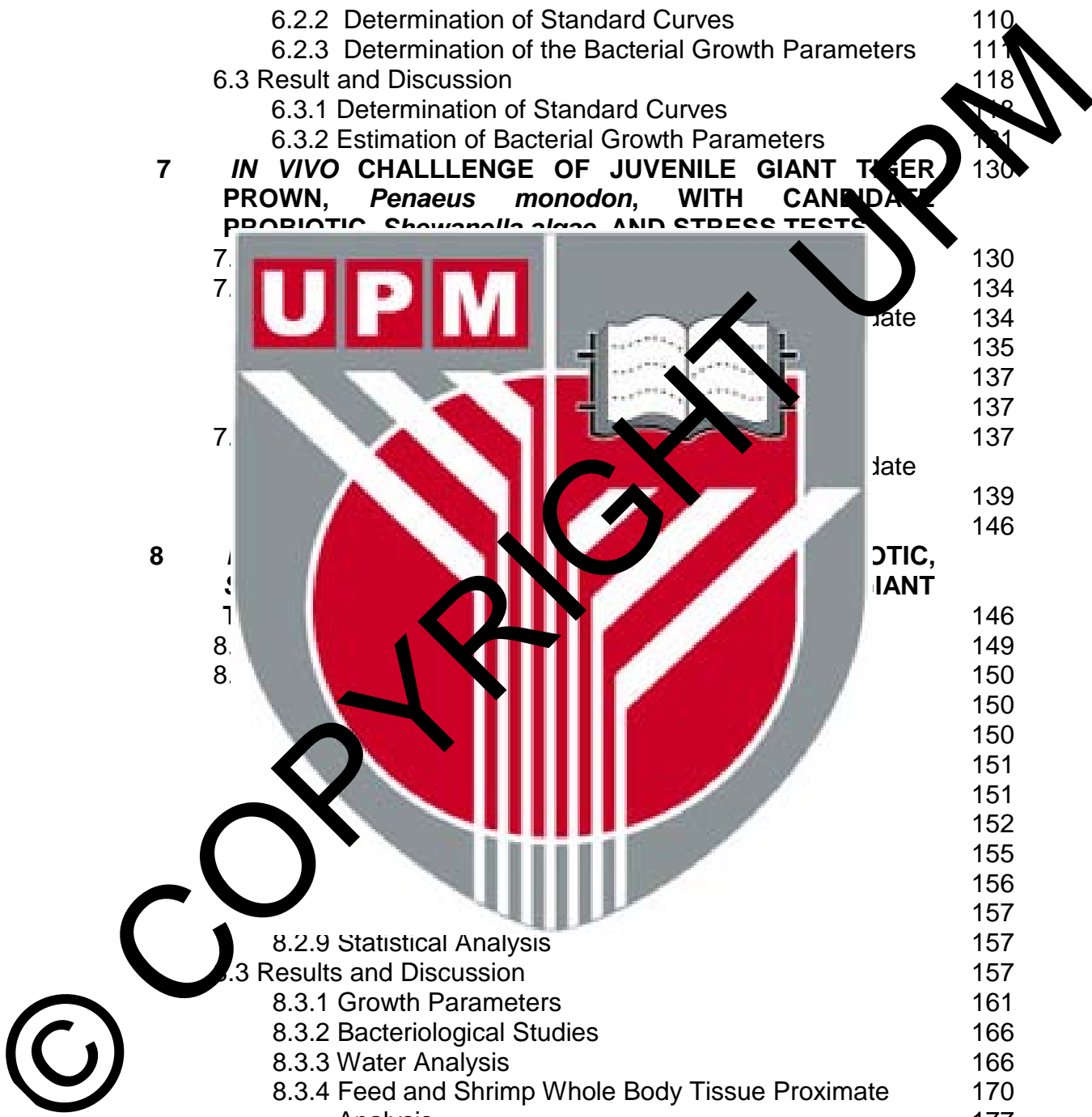
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