



UNIVERSITI PUTRA MALAYSIA

***ISOLATION AND CHARACTERIZATION OF LACTIC ACID
BACTERIA FROM GASTROINTESTINAL TRACT OF SNAKEHEAD
(Channa striatus, Bloch) AS PROBIOTIC FOR FRESHWATER FISH***

SEYED KAMALEDDIN ALLAMEH

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FOR FRESHWATER FISH**

BY

SEYED KAMALEDDIN ALLAMEH

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Doctor of Philosophy**

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In the name of God

“From water everything is alive”

(Quran, Sourah: Al-Anbiya, Vers: 30)

DEDICATION

To all teachers and researchers



Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Associate Professor Hassan bin Hj. Mohd. Daud, PhD

Faculty/Institute: Institute of Bioscience

The intensive application of antibiotics to prevent and control the bacterial diseases in aquaculture has resulted in development of antibiotic-resistant bacteria. Therefore, probiotics as an alternative strategy have been suggested to be used as replacement for antimicrobial drugs and growth promoters. Lactic acid bacteria (LAB) constitute a group of G+ve bacteria with a high ability to produce antibacterial compounds and improve fish performance which makes them excellent probiotics. In the present study, snakehead (*Channa striatus*), an indigenous commercial freshwater fish in Southeast Asia countries was chosen for the isolation of LAB. Isolates obtained on de Man Rogosa and Sharp (MRS) agar and broth showed 27 and 9 pure isolates from the fingerling and adults fishes, respectively. According to the antagonistic plate test against *Aeromonas hydrophila* as a main freshwater fish pathogen, five LABs i.e. three isolates from the fingerlings and two from the adults that showed the greatest inhibition zone were selected. The selected LABs were identified as *Aerococcus*

viridans, *Lactobacillus delbreuckii* sp. *delbreuckii* and *Enterococcus faecalis* from fingerlings and *Lactobacillus fermentum* and *Leuconostoc mesenteroides* sp. *mesenteroides* from adult fish. Probiotic property evaluation of LAB candidates showed that they could survive and grow at pH 3 up to 8 ($P < 0.05$). They could also tolerate bile salt concentrations from 0.0 to 0.3% ($P < 0.05$). The LABs were active at different levels of NaCl (0.0 to 4%) and also, at various temperatures ranging from 15 °C to 45 °C, but showed no growth at 10 °C and 50 °C. Antagonistic effects against three tested fish pathogens i.e. *A. hydrophila*, *Pseudomonas aeruginosa* and *Shewanella putrefaciens* indicated that *En. faecalis*, *L. fermentum* and *Leu. mesenteroides* sp. *mesenteroides* had the highest inhibition activities. The antibiotic sensitivity test showed that *En. faecalis* had more antibiotic's resistance property against some antibiotics as compared to other LAB ($P < 0.05$). According to probiotic characterization as screening, *En. faecalis*, *L. fermentum* and *Leu. mesenteroides* were selected for *in vivo* experiments. The viability of LABs in feed stored at 4 °C was higher than 25 °C during four weeks storage. Significant increased of the LAB proportion was observed in the fish intestine fed LAB-fortified diets as compared to the control group ($P < 0.05$). In addition, the lower G-ve bacteria population in the fish intestine was observed for experimental groups as compared to the control group. Effects of mono and multi-species of LAB candidates on the body composition of Javanese carp (*Puntius gonionotus*) indicated that LAB-fortified diets could not seriously affect the chemical composition of experimental fish carcasses. The survivability was the same for all experimental groups. The concentration of 10^7 cfu/g diets was overall more effective than 10^5 and 10^9 cfu/g diets. The use of *En.*

faecalis as probiotic had more positive effects than *L. fermentum* and *Leu. mesenteroides* on growth, feed conversion ratio, specific growth rate, and protein efficiency ratio ($P < 0.05$). Moreover, the use of *En. faecalis* resulted in an increase in the production of protease and lipase enzymes in the digestive tract of Javanese carp. Results obtained from the short chain fatty acid determination which included acetate, propionate and butyrate showed that treated group with *En. faecalis* could significantly ($P < 0.05$) enhance the propionate and butyrate production as compared to the control. An *in vivo* challenge test of *A. hydrophila* as a fish pathogen with *En. faecalis* as a probiotic on Javanese carp indicated that this probiotic could protect the fish against *A. hydrophila* and showed a higher survivability compared to the control. The presence of *En. faecalis* in the diet could affect immune response to enhance the antibody level as a humoral response. The fish treated with a LAB-fortified diet (*En. faecalis*) and added pathogen (*A. hydrophila*) into the water showed the highest antibody level as compared to the control group ($P > 0.05$). In conclusion, LABs can be normal microbiota in the gastrointestinal tract of the snakehead. In addition, the use of *En. faecalis* as a probiotic had positive effects on overall fish performance, digestive enzymes and short chain fatty acid production, survivability in challenge with pathogen and increased humoral immune response in Javanese carp. This LAB as an environmental friendly agent can be suggested as an alternative to antibiotics in freshwater fish culture.

Abstrak tesis untuk dikemukakan kepada Senat, Universiti Putra Malaysia bagi memenuhi keperluan ijazah Doktor Falsafah

**PEMENCILAN DAN PENCIRIAN BAKTERIA ASID LAKTIK DARI
SALURAN GASTROUSUS IKAN HARUAN (*Channa striatus*, Bloch 1793)
SEBAGAI PROBIOTIK UNTUK IKAN AIR TAWAR**

Oleh

SEYED KAMALEDDIN ALLAMEH

Jun 2012

Pengerusi: Prof. Madya Hassan bin Hj. Mohd. Daud, PhD

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Penggunaan antibiotik secara intensif bagi mengelak dan merawat penyakit bakteria dalam akuakultur telah menyebabkan terjadinya bakteria yang rentang antibiotik. Oleh itu probiotik adalah strategi alternatif yang telah disyorkan sebagai pengganti kepada dadah antimikrobial, penggalak pertumbuhan dan peransang imuniti. Bacteria asid laktik (BAL) terdiri dari kumpulan bakteria G+ve yang mempunyai ciri morfologikal, metabolik dan fisiologi yang menjadikan mereka probiotik yang amat baik. Di dalam kajian ini, ikan haruan (*Channa striatus*), sejenis ikan tempatan berkomersial di negara Asia Tenggara telah dipilih untuk pemencilan BAL. Percambahan koloni di atas agar *de Man Rogosa and Sharp* (MRS) dan kaldu memperolehi 27 isolat tulin dari ikan bersaiz jari dan sembilan dari ikan dewasa. Keputusan menunjukkan beberapa BAL yang lazim dalam perut dan saluran usus kedua-dua saiz ikan. Mengikut ujian plat antagonis melawan *Aeromonas hydrophila*

sebagai patogen ikan, lima BAL yakni tiga isolat dari ikan bersaiz jari dan dua dari ikan dewasa menunjukkan zon kesekatlakuan terbesar. Isolat tersebut dikenalpasti sebagai *Aerococcus viridans*, *Lactobacillus delbreuckii* sp. *delbreuckii* dan *Enterococcus faecalis* dari ikan bersaiz jari, dan *Lactobacillus fermentum* dan *Leuconostoc mesenteroides* sp. *mesenteroides* dari ikan dewasa. Penilaian sifat-sifat calon probiotik BAL menunjukkan bahawa mereka boleh hidup dan tumbuh pada pH 3 sehingga pH 8 ($P < 0.05$). Mereka juga boleh tahan dalam garam hempedu dari kepekatan 0.0 ke 0.3% ($P < 0.05$). BAL aktif pada beberapa paras NaCl berlainan (0.0 to 4%) dan juga pada suhu berjulat dari 15 °C to 45 °C, tetapi tiada pertumbuhan pada 10 and 50 °C. Kesan antagonis terhadap tiga patogen ikan iaitu *A. hydrophila*, *Pseudomonas aeruginosa* and *Shewanella putrefaciens* menunjukkan *En. faecalis*, *L. fermentum* dan *Leu. mesenteroides* sp. *mesenteroides* mempunyai aktiviti kesekatlakuan tertinggi. Ujian kepekaan antibiotic menunjukkan *En. faecalis* mempunyai lebih banyak kerentangan terhadap beberapa antibiotik dibandingkan dengan LAB lain (keertian pada $P < 0.05$). Berdasarkan pencirian probiotik, *En. faecalis*, *L. fermentum* and *Leu. mesenteroides* telah dipilih untuk eksperimen *in vivo*. BAL terpilih dengan kesan keertian ($P < 0.05$) menunjukkan kebolehidupan tinggi dalam diet disimpan pada 4 °C and 25 °C. Tambahan lagi, BAL juga dapat merendah percambahan populasi bakteria G-ve dalam saluran gastrousus. Pertambahan yang bererti BAL dapat dilihat dalam usus ($P < 0.05$). Kesan calon BAL secara tunggal dan pelbagai terhadap komposisi badan ikan lampam jawa (*Puntius gonionotus*) menunjukkan bahawa diet yang ditambah dengan BAL tidak memberi kesan jelas terhadap ke atas komposisi kimia karkas ikan ujian. Selain itu tiada kematian yang

dilihat. Oleh itu BAL yang dipilih adalah tidak berbahaya dan tidak memberi kesan sampingan terhadap ikan ujian. Diet berkepekatan 10^7 cfu/g pada keseluruhannya adalah lebih efektif dari kepekatan 10^5 dan 10^9 cfu/g. Walaubagaimana pun, data yang diperolehi menunjukkan penggunaan *En. faecalis* sebagai probiotik memberi lebih kesan positif dari *L. fermentum* and *Leu. mesenteroides* terhadap pertumbuhan, nisbah pertukaran makanan, kadar pertumbuhan tentu dan nisbah kecekapan protein (keertian pada $P < 0.05$). Tambahan lagi, penggunaan *En. faecalis* sebagai probiotik menyebabkan peningkatan pengeluaran enzim protease dan lipase dalam saluran penghadaman ikan lampam jawa (*P. gonionotus*). Juga bakteria ini lebih efektif merembeskan enzim protease dari lipase. Keputusan dari penentuan asid lemak berantai pendek termasuk asetat, propionat dan butirat menunjukkan kumpulan yang dirawat dengan *En. faecalis* boleh, secara bererti ($P < 0.05$) meningkatkan pengeluaran propionat and butirat berbanding dengan kawalan. Ujian cabaran *in vivo* *A. hydrophila* sebagai patogen ikan dengan *En. faecalis* sebagai probiotik ke atas lampam jawa menunjukkan probiotik ini boleh melindungi dari jangkitan dan menunjukkan kesan penghalang yang efektif terhadap patogen ini. Keputusan dari gerak balas sistem imun menunjukkan kehadiran *En. faecalis* dalam diet boleh meransang gerak balas peningkatan paras antibodi sebagai keimunan humoral. Kumpulan ikan dengan diet yang ditambah dengan BAL dan bersama patogen (*A. hydrophila*) dalam air menunjukkan paras antibodi tertinggi berbanding dengan kawalan ($P > 0.05$). Pada kesimpulannya, BAL boleh menjadi mikrobiota normal dalam saluran gastrousus ikan haruan. Tambahan lagi, penggunaan *En. faecalis* sebagai probiotik memberi kesan positif ke atas keseluruhan perkembangan ikan,

enzim pencernaan dan pengeluaran asid lemak berantai pendek, kemandirian dalam cabaran dengan patogen dan peningkatan ransangan imun humoral di lampam jawa. Bakteria asid laktik ini sebagai satu agen yang mesra alam boleh disyorkan sebagai alternatif kepada penggunaan antibiotik dalam kultur ikan air tawar.



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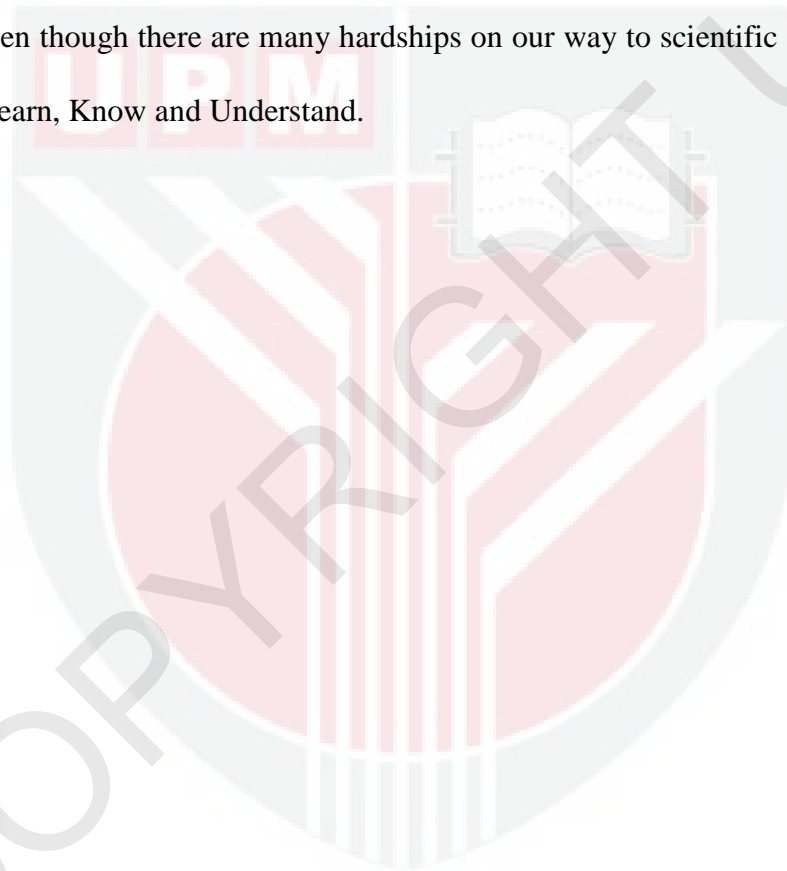
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I certify that a Thesis Examination Committee has met on 20 June 2012 to conduct the final examination of Seyed Kamaleddin Allameh on his thesis entitled “Isolation and Characterization of Lactic Acid Bacteria from Gastrointestinal Tract of Snakehead (*Channa striatus*, Bloch) as Probiotic for Freshwater Fish” 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 Doctor of Philosophy.

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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 other institutions.



SEYED KAMALEDDIN LLAMEH

Date: 20 June 2012

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