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ISOLATION OF BENEFICIAL BACTERIA AND FEED INCORPORATED WITH BENEFICIAL BACTERIA ON GROWTH PERFORMANCE OF ASIAN SEA BASS (Lates calcarifer, BLOCH) FINGERLING

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By

WENDY FERRINA ANAK TARRY

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

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

ISOLATION OF BENEFICIAL BACTERIA AND FEED INCORPORATED WITH BENEFICIAL BACTERIA ON GROWTH PERFORMANCE OF ASIAN SEA BASS (*Lates calcarifer*, BLOCH) FINGERLING

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Chair: Abu Hena Mustafa Kamal, PhD

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The intestinal tract of healthy animals is assumed to be the natural place of 'good' micro-organisms. The present study investigated different parts of intestine of Asian sea bass (*Lates calcarifer*, Bloch) to isolate and identify strains potentially useful for fish probiotics. *In vivo* test was done to validate the performance of isolated *Enterobacter ludwigii* potential probiotic to be used in sea bass fry rearing in aquaculture industry. Five Randomized Complete Block Design (RCBD) treatments for different concentration of *E. ludwigii* were mixed with sea bass feed, i.e., T1 (control; without *E. ludwigii*), T2 (1×10^1 cfu/g of *E. ludwigii*), T3 (1×10^3 cfu/g of *E. ludwigii*), T4 (1×10^6 cfu/g of *E. ludwigii*), T5 (1×10^9 cfu/g of *E. ludwigii*) and fed to the sea bass fry for 28 days. The total culturable aerobic gut bacteria of both live and dead sea bass samples ranged from $1.17-84.00 \times 10^6$ cfu/g, with counts being higher in posterior intestine ($1.97-84.00 \times 10^6$ cfu/g) compared to the number of cells occurring in anterior ($0.21-7.87 \times 10^6$ cfu/g) and middle ($1.17-3.50 \times 10^6$ cfu/g) parts, although significantly ($p<0.05$) higher numbers were associated with live fish. The isolate MS32 was selected that inhibit the growth of fish pathogens *Vibrio parahaemolyticus* and *Aeromonas hydrophilla* in disc diffusion, well diffusion assay and cross streak. The isolate MS32 produced gamma-hemolysin and was identified by standard biochemical tests and 16S rDNA sequences as *E. ludwigii*. The species *E. ludwigii* can be grouped as moderately halophile marine bacteria. Storage at 4^oC is suitable to store sea bass feed pellet that have been mixed with *E. ludwigii* and can be used for about 20 days.

In vitro test showed that *E. ludwigii* formed a clear inhibition zone against 3 fish pathogens at concentration level of 1×10^9 cfu/ml via well and disc diffusion method. Presence of *E. ludwigii* in feed was safe to be used to the sea bass fry where no significant difference ($p>0.05$) among treatments and control. *E. ludwigii* did not improved the growth or increased appetite of sea bass fry with no significant difference ($p>0.05$) on the growth performance of sea bass fry. The number of *E. ludwigii* in the gastrointestinal tract of sea bass fry and each water sample were also not significantly different ($p>0.05$). Significant difference ($p<0.05$) in nitrate, ammonium and phosphate concentrations of rearing water of sea bass fry was observed within the treatments. The concentration of nitrate range from 0.0012–0.0022 mg/L, phosphate concentration from 0.032–0.045 mg/L and ammonium concentrations from 0.981–1.457 mg/L. The presence of *E. ludwigii* can help to improve the water quality compared to control group. Observation after 7 days of challenge test against *V. parahaemolyticus* (2×10^8 cfu/ml) showed that the highest survival percentage (100%) was found for T5 compared to T1 (79%).The findings of the study revealed that *E. ludwigii* could be a potential probiotics for rearing of sea bass fry where improvement of water quality in the rearing tank of sea bass fry and protection against fish pathogen were remarkable.

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

PENGASINGAN BAKTERIA BERFAEDAH DAN PENGATURAN MAKANAN DENGAN BAKTERIA BAIK TERHADAP PRESTASI PERTUMBUHAN BENIH SIAKAP ASIA (*Lates calcarifer*, BLOCH)

Oleh

WENDY FERRINA ANAK TARRY

Januari 2015

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Saluran perut dan usus haiwan yang sihat adalah tempat semulajadi bagi mikroorganisma baik. Kajian ini adalah untuk mengkaji bahagian yang berbeza pada saluran perut dan usus ikan Siakap (*Lates calcarifer,* Bloch) untuk pengasingan dan mengenalpasti strain yang berpotensi menjadi probiotik yang berguna kepada ikan. Kajian *in vivo* telah dijalankan untuk memastikan strain *Enterobacter ludwigii* sebagai probiotik yang berpotensi untuk digunakan dalam penternakan benih siakap dalam industri akuakultur. Lima rawatan secara 'Randomized Complete Block Design (RCBD)' dengan kepekatan *E. ludwigii* yang berbeza telah dicampur dengan makanan siakap, T1 (kawalan; tanpa *E. ludwigii*), T2 (*E. ludwigii* dengan 1×10^1 cfu/g), T3 (*E. ludwigii* dengan 1×10^3 cfu/g), T4 (*E. ludwigii* dengan 1×10^6 cfu/g), T5 (*E. ludwigii* dengan 1×10^9 cfu/g) dan diberikan kepada benih siakap selama 28 hari. Jumlah bakteria aerobik perut dan usus yang terdapat pada ikan segar dan ikan mati adalah dalam lingkugan $1.17-84.00\times10^6$ cfu/g, dimana kiraan yang tertinggi adalah pada bahagian posterior usus ($1.97-84.00\times10^6$ cfu/g) berbanding kiraan pada anterior usus ($0.21-7.87\times10^6$ cfu/g) dan bahagian tengah ($1.17-3.50\times10^6$ cfu/g), dimana dengan perbezaan ketara ($p<0.05$) kiraan yang tertinggi adalah pada ikan segar. Strain MS32 dipilih dimana dapat menghalang pertumbuhan patogen ikan *Vibrio parahaemolyticus* dan *Aeromonas hydrophilla* melalui cakera serapan, serapan telaga-agar and goresan melintang. MS32 membentuk gamma-hemolisis dan dikenalpasti sebagai *E. ludwigii* menerusi ujian biokimia dan 16S rDNA. Spesis *E. ludwigii* boleh dikelaskan kepada bakteria sederhana 'halophile' marin. Pelet siakap yang telah dicampurkan dengan *E. ludwigii* sesuai disimpan pada suhu 4^oC dan tempoh penggunaannya selama 20 hari sahaja.

Kajian *in vitro*, *E. ludwigii* telah membentuk zon perencatan pertumbuhan terhadap 3 patogen ikan pada kepekatan 1×10^9 cfu/ml melalui kaedah cakera serapan dan serapan telaga-agar. Kehadiran *E. ludwigii* dalam makanan adalah selamat digunakan untuk benih siakap dimana tiada perbezaan ketara ($p>0.05$) diantara kumpulan rawatan dan kawalan. *E. ludwigii* tidak meningkatkan prestasi pertumbuhan atau selera benih siakap dimana tiada perbezaan ketara ($p>0.05$) bagi prestasi pertumbuhan benih siakap. Pengiraan *E. ludwigii* pada saluran perut dan usus benih siakap serta sampel air masing-masing adalah tiada perbezaan ketara ($p>0.05$). Perbezaan ketara ($p<0.05$) didapati pada kepekatan nitrat, ammonium dan fosfat dalam sampel air rawatan yang diggunakan untuk menternak benih siakap. Kepekatan nitrat adalah di antara 0.0012–0.0022 mg/L, fosfat di antara 0.032–0.045 mg/L dan ammonium antara 0.981–1.457 mg/L. Kehadiran *E. ludwigii* dapat membantu memperbaiki kualiti air jika dibandingkan dengan rawatan kawalan. Pemerhatian selama 7 hari semasa ujian cabaran terhadap *V. parahaemolyticus* (2×10^8 cfu/ml) menunjukkan kadar yang hidup adalah tinggi (100%) pada T5 berbanding dengan T1 (79%). Penemuan kajian ini mengesahkan bahawa *E. ludwigii* boleh menjadi probiotik yang berpotensi untuk membantu dalam penternakan benih siakap dimana peningkatan kualiti air dalam tangki ternakan benih siakap dan perlindungan daripada patogen ikan telah diperhatikan.

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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 of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

RCBD	-	Randomized complete block design
SCT	-	Salinity Conductivity and Temperature meter
TDS	-	Total dissolved solid
SGR	-	Specific growth rate
HIS	-	Hepatosomatic index
VSI	-	Verosomatic index
CI	-	Condition index
FCR	-	Feed conversion rate
PI	-	Protein intake
PER	-	Protein efficiency rate
DO	-	Dissolved oxygen
OD	-	Optical density
MA	-	Marine agar
MB	-	Marine broth
TCBS	-	Thiosulphate citrate bile sucrose
MSA	-	Market siakap anterior
MSM	-	Market siakap middle
MSP	-	Market siakap posterior
LSA	-	Live siakap anterior
LSM	-	Live siakap middle
LSP	-	Live siakap posterior
EDTA	-	Ethylene diamine tetraacetic acid

CHAPTER 1

INTRODUCTION

Sea bass is a commercially important coastal, estuarine and freshwater farmed species in the Indo-Pacific region. The culture of this species has extended recently in North America and Europe (Katersky and Carter, 2007). This fish is popular for its delicately-flavored flesh and high market price. It has also fast growth rate and grows to a larger size, thus making it very suitable for aquaculture (Cheong, 1990). In recent years, the culture of sea bass both in pond and cage are in moribund stage due to feeding cost and feed available in the Southeast Asia.

Besides, disease outbreak especially the bacterial diseases has lead to the economic losses in the fish farming (Marzouk *et al.*, 2008). Disease can cause fish mortality and in other hand increase the expenses for treatment, postponement or loss opportunity to sell the fish (Adedeji *et al.*, 2011). The usage of antibiotics to control disease can affect human health and also environment (Birkbeck, 2004). On the other hand, the natural stocks of this fish species have drastically reduced due to natural and manmade catastrophes, degradation of aquatic environment due to climate change and the reduction of many wetlands and water areas in Asia including Malaysia. These factors have created a serious problem to their breeding, migration route, availability and stock, and thus assume this fish become gradually taking place in the list of endangered species.

According to IUCN, there is still data deficiency for this fishery species all over the world. In order to maintain this fish population as well as to conserve their diversity, development of suitable feed component for the rearing and growing of sea bass is very essential both in hatchery, nursery and pond systems. There are also small numbers of sea bass seed from local market with low quality. This may hinder the sustainable production of marine fish culture including sea bass in Malaysia. The use of trash fish as fish feed leads to the breakage of diseases. However, it is difficult to change to pellet where the price is expensive and not readily available compared to trash fish. The mass mortality is also noticed, which is related to the water quality and oxygen depletion elsewhere (Othman, 2008). Studies have been conducted on nutrition, carbohydrate and lipid variation on growth and protein synthesis, feeding frequency on the growth for sea bass (Cuzon *et al.*, 1989; Catacutan and Coloso, 1997; Katersky and Carter, 2007; Biswas *et al.*, 2011) elsewhere, however, no systematic information are available on the diets with bacterial products of this important fishery.

Several studies are conducted on the important roles of probiotics in aquaculture related to the productivity and nutrition of cultured animals, modification of host-associated/ambient microbial community and prevention of diseases and/or improvement of water quality (Verschuere *et al.*, 2000; Wang *et al.*, 2008; Abu Hena *et al.*, 2011). In general, probiotics is harmless to the host and also human being while improved disease resistance for host against diseases. The usage of probiotics is an eco-

friendly alternative measure for sustainable aquaculture (Sihag and Sharma, 2012). Probiotics can help to increase the resistance of host against pathogen, improve their digestion and absorption, and improve feed nutrition as well as maintaining and improving water quality in culture system (Havenaar and Huis, 1992; Gatesoupe, 1999; Verschuere *et al.*, 2000; Cruz *et al.*, 2012).

The isolation and screening of the potential probiotics was done from the gastrointestinal tract of the juvenile sea bass where usually, fish gastrointestinal tract have a high and wide population diversity of microbiota that is mainly dominated by bacteria (Spanggaard *et al.*, 2001; Pond *et al.*, 2006; Denev *et al.*, 2009). The intestinal microbiota is very important since they have their own and specific metabolic, trophic and protective function (Denev *et al.*, 2009; Guarner and Malagelada, 2003). Studies revealed that bacterial products such as probiotics could be useful for both the food and biological control agents of fish disease and activities on the rates of nutrient regeneration for culture organisms (Yasuda and Taga, 1980), hence this study is initiated to investigate on marketed and wild live sea bass from Sarawak region.

The suitable doses of the potential probiotics for the usage towards sea bass culture are an important factor that will influence their effectiveness. A consideration for an appropriate dosage level should be known because it depends on the probiotics species, host fish species, rearing conditions and specific goal of feeding application (Merrifield *et al.*, 2010). The feeding trial by potential probiotics was conducted using sea bass fry since their gastrointestinal tract is still sterile and not fully developed. Early development stages are suitable to apply probiotic treatments since they are highly exposed to gastrointestinal microbiota-associated disorder, because they still have incomplete immune system and their digestive tract is not fully developed (Timmermans, 1987; Vadstein, 1997; Gatesoupe, 1999).

Objectives

The objectives of the study are;

1) to isolate the beneficial bacteria (probiotics) from the gastrointestinal tracts of marketed and live sea bass, *Lates calcarifer* (Bloch) and;
2) to observe the growth performance and survival of sea bass fry fed with isolated potential beneficial bacteria strain (*E. ludwigii*).

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