PROBIOTIC EFFECTS OF ISOLATED *Bacillus* sp. ON *ARTEMIA*

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THIS DISSERTATION IS SUBMITTED AS A PARTIAL FULFILLMENT OF THE REQUIREMENTS TO GRADUATE AS A BACHELOR OF SCIENCE WITH HONOURS

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DECLARATION

I declare that this dissertation is the results of my own independent work, except where otherwise stated.

31 March 2008

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ABSTRACT

Bacterial strain used in this study was isolated from sediment obtained from Sepanggar Bay. The strain was identified as *Bacillus coagulans* through some biochemical and morphological tests. This study was conducted to study probiotic effects of *B. coagulans* in live feed, especially on *Artemia*. The live feed was fed with cod oil juice (1 ml), mixture of cod oil juice and *B. coagulans*, and *B. coagulans* (1.84x10^{-3} mg/l). In control live feed was not fed and leaved starved. The study indicated that the probiotic strain improves the growth (3.58 mm) and survival (60%) of *Artemia*. *Artemia* fed with a mixture of *B. coagulans* and cod oil juice showed a growth of 3.58 mm whereas *Artemia* fed with *B. coagulans* alone showed a growth of 2.40 mm. Significantly higher growth (P<0.05) was recorded in *Artemia* fed with the mixture of probiotic and cod oil juice. *Artemia* fed with probiotic alone showed significantly higher survival (P<0.05) compared to *Artemia* fed with other feeds. The study signifies that the use of *B. coagulans* does increases the growth and survival of *Artemia* compared to control or cod oil juice. Therefore, can be concluded that *B. coagulans* has potentiality as probiotic. Hence, *B. coagulans* can be applied as probiotic for a sustainable aquaculture.
ABSTRAK

Bakteria yang diguna dalam eksperimen ini telah diasingkan dari enapan yang diambil dari Teluk Sepanggar. Bakteria tersebut telah dikenalpasti sebagai Bacillus coagulans melalui kajian biokimia dan morpologi. Eksperimen ini dijalankan untuk mengkaji kesan probiotik B. coagulans atas makanan hidup, terutamanya Artemia. Artemia telah diberi jus minyak kod (1 ml), campuran B. coagulans dan jus minyak kod serta B. coagulans (1.84x10^{-3} mg ml^{-1}). Dalam kawalan, Artemia tidak diberi makan. Eksperimen ini menunjukan strain probiotik meningkatkan pertumbuhan (3.58 mm) dan kadar hidup (60%) Artemia. Artemia dalam campuran jus minyak cod dan B. coagulans menunjukkan pertumbuhan sebanyak 3.58 mm manakala Artemia dalam B. coagulans sahaja menunjukkan pertumbuhan sebanyak 2.40 mm. Pertumbuhan Artemia adalah tinggi secara bererti \( P<0.05 \) apabila menggunakan campuran probiotik dan jus minyak kod. Kadar hidup Artemia pula menunjukkan signifikasi yang tinggi \( P<0.05 \) apabila menggunakan probiotik sahaja berbanding diet-diet yang lain. Eksperimen ini menunjukkan B. coagulans meningkatkan pertumbuhan dan kadar hidup Artemia berbanding kawalan atau jus minyak kod. Dengan ini dapat disimpulkan bahawa B. coagulans mempunyai potensi sebagai probiotik. Oleh yang demikian, B. coagulans dapat diaplikasikan sebagai probiotik dalam akuakultur yang sedang berkembang.
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CHAPTER 1

INTRODUCTION

1.1 AQUACULTURE

Aquaculture, most simply defined as the rearing or farming of aquatic species under controlled conditions, is a rapidly growing industry, accounting for over one-third of all direct fisheries consumption. It is an important economic activity in many countries and offers a number of opportunities to contribute to poverty alleviation, employment, community development, the reduction of overexploitation of natural resources, and food security in tropical and sub-tropical regions.

1.1.1 Microbes in Aquaculture

Microbes play very important and critical roles in aquaculture systems, both at hatchery and grow out level. It is said so because water quality and disease control are directly affected by microbial activity.

In hatchery facilities, the environmental conditions (availability of iron, osmotic strength, oxygen levels, pH, water quality in terms of suspended solids, and total phosphorus and temperature) and sometimes poor management practices
(inadequate nutrition, overcrowding and overfeeding) can cause stress to the organisms being cultured and thus make them more susceptible to disease outbreaks (Hansen and Olafsen, 1999; Verschuere et al., 2000; Winton, 2001). These problems can be overcome through the application of beneficial microbes.

Microbial interactions play a major role in the equilibrium between competing beneficial and potentially pathogenic micro-organisms. Microbial manipulation constitutes a viable tool to reduce or eliminate the incidence of opportunist pathogens (Balcázar, 2002). Balcázar (2002) demonstrated that the administration of a mixture of bacterial strains (Bacillus and Vibrio sp.) positively influenced the growth and survival of juveniles of white shrimp and presented a protective effect against the pathogens Vibrio harveyi and white spot syndrome virus.

Bacteria are easily introduced into cultural units through natural or artificial food sources, treated inlet water and less frequently through vertical transmission from brood stock (Alabi et al., 1997; Hansen and Olafsen, 1999; Sandaa et al., 2003; Winton 2001).

There is an increasing awareness within the aquaculture industry in the control or elimination of antibiotic use. Therefore, alternative methods need to be developed to retain a healthy microbial environment in the larval rearing tanks. One such method that is gaining acceptance within the industry is the use of probiotic bacteria to control potential pathogens.
1.2 PROBIOTICS IN AQUACULTURE

Probiotics are defined as “live microorganisms, which when are consumed in adequate amounts, confer a health benefit for the host” (Reid et al., 2003). Tannock (1997) defined probiotics as microbial cells that are administered in such a way as to enter the gastrointestinal tract and to be kept alive, with the aim of improving health.

Probiotics in other sectors have been unawarely used to preserve food, and these empirical methods contributed to improve human health (Bengmark, 1998). Microbial probiotics can influence the systemic immune systems in various ways. Firstly, they can enhance the defensive property of intestinal mucosa whose function is like a barrier against the antigens (i.e., bacterial pathogens) (Salminen et al., 1996). Currently, there is a wide range of probiotic combination products commercially available such as animal feeds, dairy foods, infant and baby foods, fruit juice-based products, and pharmaceuticals.

Probiotics have a vital role in maintaining aquatic animals in healthy condition, and may provide an alternative to the use of antimicrobial compounds (Verschuere et al., 2000).

The use of probiotics or beneficial bacteria, which enhance growth and survival besides control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment. The use of probiotics in human and animal nutrition is well documented (Fuller, 1992; Mulder et al., 1997; Rinkinen et al., 2003) and lately, they have begun to be applied in aquaculture (Gatesoupe, 1999;
Gomez-Gil et al., 2000; Verschuere et al., 2000; Irianto and Austin, 2002; Bachère, 2003).

Gatesoupe (1999) concluded that probiotics for aquaculture should be antagonistic to pathogens, colonize intestines and increase resistance of the host to pathogens.

In aquaculture the use of bacteria as probiotics and as alternatives to antibiotics are required to boost the production of aquatic animals. There are several microbial strains used as probiotics in aquaculture systems. The common probiotics used in aquaculture, belonging to Bacillus sp., Lactobacillus sp., Bifidobacterium sp., Vibrio sp., Saccharomyces sp., Enterococcus sp., Bacillus subtilis, are now being used for oral bacteriotherapy in aquaculture.

1.3 Bacillus sp. AS PROBIOTIC

Most probiotics proposed as biological control agents in aquaculture belong to the genus Bacillus, to the lactic acid bacteria, to the genus Vibrio, or to the genus Pseudomonas, although other genera or species have also been mentioned.

Bacillus sp. such as B. megaterium, B. polymyxa, B. subtilis, and B. licheniformis have been proposed and tested as probiotics in aquaculture (Gatesoupe, 1991; Vershuere et al., 2000).
The spores of *Bacillus* sp. are especially easy to introduce in dry food, and this is an additional advantage of these promising candidate probiotics (Moriarty, 1998; Queiroz and Boyd, 1998; Kennedy *et al.*, 1998; Rengpipat *et al.*, 1998; Sugita *et al.*, 1998).

*Bacillus* sp. have been successfully used as probiotics in the aquaculture of black tiger shrimp (*Penaeus monodon*) in Thailand, where there was an improvement in the growth rate (47%) and survival rate when challenged with *Vibrio harveyi* (Rengpipat *et al.*, 1998).

The actual data of Moriarty (1998) showed the inhibitory activity of *Bacillus* sp. against luminous *Vibrio* sp. in pond sediment, but the effect on prawn survival might be due either to a probiotic effect, or to an indirect effect on animal health. For instance, the degradation of organic matter by *Bacillus* sp. might improve water quality. Therefore, the use of *Bacillus* sp. as pond supplement needs further investigation to be considered as a probiotic treatment, with particular attention to a possible intestinal transience.

There is potential for application of probiotics in aquaculture; however the use of terrestrial strains of bacteria as probiotics for aquaculture has had limited success, as strain characteristics of bacteria are dependant upon the environment in which they thrive. Therefore, isolating potential probiotic bacteria from local marine environment in which they grow optimally is a better approach.
I chose *Bacillus* sp. as my targeted probiotic bacteria since *Bacillus* sp. has a lot of contribution in aquaculture field, such as increasing the growth and survival of cultured organisms, improving water quality of culture unit, and increase resistance towards pathogens. Moreover, *Bacillus* sp. can be easily isolated from degradative soil and the native environment. This initiates me to isolate *Bacillus* strain from native environment, and applied on live feed. I chose *Artemia* as the live feed since it is commercially used in aquaculture field and is suitable as carrier of probiotic.
1.4 OBJECTIVES

The main objective of this research is to observe probiotic effects of isolated *Bacillus* sp. on live feed. In order to achieve this objective, there are also specific objectives which are:

1. To isolate *Bacillus* sp. from native environment.

2. To observe some of the morphological and biochemical properties of the isolated *Bacillus* strain and do preliminary identification.

3. To observe the probiotic effects on *Artemia*. 
CHAPTER 2

LITERATURE REVIEW

2.1 PROBIOTICS

Elie Metchnikoff’s work at the beginning of this century is regarded as the first research conducted on probiotics (Fuller, 1992). He described them as “microbes ingested with the aim of promoting good health”. The same definition was modified to “organisms and substances which contribute to intestinal microbial balance” (Parker, 1974), and later by Fuller (1989) to “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. These definitions originally applied to farm animals or humans, since the first studies were carried out in these species.

Probiotics are now also being used in aquaculture and therefore, the definition may have to be modified. In aquatic animals, not only the digestive tract is important but also the surrounding water. Gatesoupe (1999) defines probiotics as “microbial cells that are administered in such a way as to enter the gastrointestinal tract and to be kept alive, with the aim of improving health”. Gram et al. (1999) broadened the definition by removing the restriction to the improvement to the intestine: “a live
microbial supplement which beneficially affects the host animal by improving its microbial balance.”

The term probiotics is generally used to denote bacteria that promote the health of other organisms. Lilley and Stillwell (1965) described them as substances secreted by one microorganism, which stimulated the growth of another. An expert with the Joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), stated that probiotics are live micro-organisms, which when consumed in adequate amounts, confer a health benefit for the host (FAO/WHO, 2001).

The probiotics were defined as live microbial feed supplements that improve health of man and terrestrial livestock. The first probiotics tested in fish were commercial preparations devised for land animals. Though some effects were observed with such preparations, the survival of these bacteria was uncertain in aquatic environment. Most attempts to propose probiotics have been undertaken by isolating and selecting strains from aquatic environment. These microbes include Bacillus sp., Vibrionaceae, pseudomonads, lactic acid bacteria and yeasts.

Probiotics are a cultured product or live microbial feed supplement, which beneficially affects the host by improving its intestinal balance and health of the host. The first probiotic discovered long time ago was Lactobacillus sp., the lactic acid producing bacteria. Presently the range of probiotics extends well beyond the Lactobacillus sp. to include Bacillus sp., Vibrio sp., Pseudomonas, yeast and algae.
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