UNIVERSITI PUTRA MALAYSIA

EFFECT OF PROBIOTIC (BACILLUS SUBTILIS) IN MACROBRACHIUM ROSENBERGII CULTURE

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ABSTRACT

A study on the effect of *Bacillus subtilis* on *Macrobrachium rosenbergii* culture was conducted on July 2012 until October 2012 at Wet Laboratory, Block A, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor. The aim of this study was to determine the effectiveness of probiotic, *Bacillus subtilis* on growth performance of *Macrobrachium rosenbergii* post larvae. The post larvae of *M. rosenbergii* with an initial size range between 1.2 to 1.5 cm per post larva and stocking density of 25 post larva per aquaria was used. The experiment was carried out with three different treatments which consisted of T1 (control), T2 (commercial pellet feed-10^7 CFU *Bacillus subtilis*) and T3 (commercial pellet feed-10^{10} CFU *Bacillus subtilis*). Each treatment was replicated four times and was conducted in recirculation system in glass aquaria for 12 weeks. The data was analyzed using One-Way ANOVA by SPSS (Statistical Package for Social Science) software. The result of growth performance and survival from this experiment showed that *M. rosenbergii* post larvae treated with diet T3 significantly different ($p<0.05$) compared to other treatments.

ABSTRAK

Satu kajian ke atas kesan probiotik, *Bacillus subtilis* terhadap kultur *Macrobrachium rosenbergii* telah dijalankan pada bulan Julai 2012 sehingga Oktober 2012 di Makmal Basah, Blok A, Fakulti Pertanian, Universiti Putra Malaysia (UPM), Serdang, Selangor. Tujuan kajian ini ialah untuk menentukan keberkesanan probiotik, *Bacillus subtilis* terhadap prestasi pertumbuhan post larva *M. rosenbergii*. Saiz awal post larva *M. rosenbergii* ialah antara purata 1.2 hingga 1.5 cm setiap post larva dan kepadatan sebanyak 25 ekor post larva setiap akuarium telah digunakan. Eksperimen ini melibatkan tiga rawatan berlainan yang terdiri daripada T1 (kawalan), T2 (komersil pellet-10^7 CFU *Bacillus subtilis*) dan T3 (komersil pellet-10^{10} CFU *Bacillus subtilis*). Setiap rawatan dibahagikan kepada empat replikasi dan dijalankan selama 12 minggu. Data telah dianalisis menggunakan One-Way ANOVA oleh SPSS (program statistic sains sosial). Keputusan prestasi pertumbuhan dan kadar hidup yang didapati daripada eksperimen ini menunjukkan post larva yang dirawat dengan diet T3 mempunyai perbezaan beerti ($p<0.05$) jika dibandingkan dengan kesemua rawatan.

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LIST OF ABBREVIATIONS AND SYMBOLS

Cm	Centimeter
Ml	Milimeter
0C	Degree Celcius
G	Gram
%	Percentage
pH	Measure of acidity
DO	Dissolved oxygen
mg/L	Miligram per liter
CFU	Colony forming unit
SGR	Specific growth rate
ANOVA	Analysis of variance
DMRT	Duncan's Multiple Range Test
SD	Standard deviation

CHAPTER 1

INTRODUCTION

Aquaculture industry is one of the rapidly growing industries in many developed and developing countries. There has been a global expansion of freshwater prawn farming since year 1995 because of the massive production in Asian countries mainly from China in the last few years (New, 2005). According to FAO (2010), world production of *Macrobrachium rosenbergii* has exceeded 400,000 tons in 2010 compared to early 1990's production. In Malaysia, this species that belongs to genus *Macrobrachium* is said to be increasingly popular among consumers (New, 2005). This impressive industry has been linked considerably with utilization of prophylactic antibiotics for disease prevention (Le and Munekage, 2004). The excess use of these antibiotics has led to antibiotic resistance to some bacterial pathogens (Venkat *et al.*, 2004). However, the safety of food consumption in recent years has become an issue by consumers. Therefore, the limitation of the use of antibiotic has brought the industry into the application of probiotics as an alternative method application.

The word probiotics is constructed from the Latin word pro (for) and the Greek word bios (life). While the definition of a probiotic was differ greatly depending of the sources through many years. (Fuller, 1992) defined probiotics as live microbial feed supplements which beneficially affect the host by improving intestinal microbial balance and subsequently increases the growth rate. Probiotics

are beneficial microorganisms which protect the host against any disease infections (Saad *et al.*, 2009). According to Kumar *et al.* (2006), probiotics is the certain microbes that play a very important role in aquaculture industry to regulate directly the water quality and disease control.

Generally, the application of probiotics can be applied directly in water tanks and ponds or encapsulated in feed through live feed (Einar, 2002). The application through injection is also possible (Austin *et al.,* 1995). They act as biocontrol agent against disease infections caused by pathogenic bacteria. The application of probiotics in aquaculture practices, are mainly used in feed formulations which has been given considerable attention (Verschurere *et al.*, 2000). Probiotics have shown its effectiveness in many types of species for growth performance, increase in immunity and survival rate (Saad *et. al.,* 2009; Merrifield *et. al.,* 2010). The most common types of probiotics used in aquaculture belong to genus, *Saccharomyces, Clostridium, Lactobacillus, Bacillus, Enterococcus, Shewanella, Leuconostoc, Carnobacterium, Aeromonas, Lactococcus* and others (De Rodriganez *et. al.*, 2009). Einar (2002) reported that, the most common bacteria used as probiotics in fish are *Vibrio* and *Pseudomonas* while *Bacillus* is used for shrimp culture.

Bacillus subtilis is a Gram-positive bacteria with unicellular rod-shaped and spore forming bacteria. It can be naturally found in soil and vegetation. It forms the colonies that are dull and wrinkled with cream to brown color. It can be arranged in singles or chains. It also has a rigid cell wall structure outside the cell which

composed of peptidoglycan, also known as murein in bacteria. It also can produce endospores which can be widespread. *Bacillus subtilis* bacteria have flagella for motility. *Bacillus subtilis* grow in the range of mesophilic temperature by normal temperature is 25-35 ^{0}C (Schaechter, 2006). The bacteria able to grow anaerobically (Bandow, 2002). *B. subtilis* is also found in several commercially fermented food products such as soybeans fermented of *B. subtilis natto*.

1.1 Statement of problems

Bacillus is known to improve the growth performance, survival, disease resistance and immunity of aquatic animals have been published (El-Dakar *et al.,* 2007), but still there is lack of knowledge about the effect of *Bacillus subtilis* on growth performance of *Macrobrachium rosenbergii* culture. Other researchers used *Lactobacillus sporogenes* and *L. Acidophilus in Macrobrachium rosenbergii* culture. Probiotics are reported able to enhance growth, immunity, survival rate and nutrition of host organisms. Thus, this study investigated and identified the optimal concentration of *B. subtilis* to be sprayed onto commercial feed for attaining good survival and growth in *M. rosenbergii* post larvae.

1.2 Objective

The objective of this study is:

1. To determine the effectiveness of probiotic, *Bacillus subtilis* on growth performance of *Macrobrachium rosenbergii* post larvae.

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