UNIVERSITI PUTRA MALAYSIA

CONTROL OF Vibrio alginolyticus IN Artemia CULTURE BY TREATMENT WITH BACTERIAL PROBIOTICS

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This project report is submitted in partial fulfillment of the requirements for the degree of Bachelor of Agriculture (Aquaculture)

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CERTIFICATION OF APPROVAL

DEPARTMENT OF AQUACULTURE

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This is to certify that I have examined the final project report and all corrections have been made as recommended by the panel of examiners. This report complies with the recommended format stipulated in the AKU4999 project guidelines, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia.

Signature and official stamp of supervisor/& cosupervisor:

_____

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ABSTRACT

Probiotics are bacteria which can act as health promoter of other organism by modifying the ambient microbial community, enhancing feed nutritional value, enhancing the host response towards disease, or improving the quality of its ambient environment. Probiotics *Micrococcus* spp. (JAQ07) and *Bacillus* spp. (JAQ04) were used as potential probiotics in this experiment. Both probiotics were identified as Gram-positive with different morphology. *Micrococcus* spp. was a rod-shaped bacterium whereas *Bacillus* spp. was cocci-shaped bacterium. In *in-vivo* assay, Artemia was used as a host and treated with *Bacillus* spp. and *Micrococcus* spp. at different concentrations (10^2, 10^4 and 10^6 CFU ml^{-1}) and then challenged with *Vibrio alginolyticus* at concentration 10^5 CFU ml^{-1}. *Bacillus* spp. able to enhance the survival of Artemia better compared with *Micrococcus* spp. when challenged with *Vibrio alginolyticus.* Artemia treated with *Bacillus* spp. at concentration of 10^6 CFU/ml and challenged with *Vibrio alginolyticus* showed 70% of survival compared with the survival of challenged Artemia with *Vibrio alginolyticus* only (20% survival rate) after 7 days. Meanwhile Artemia pre-incubation with *Micrococuss* spp. and challenged with *Vibrio alginolyticus* showed 68% survival. Both probiotics are not harmful because no significant of survival was found compared to the control. *Micrococcus* spp. was able to slightly reduce the vibrios load in Artemia and culture water. However, *Bacillus* spp. was not able to reduce the vibrios load in water culture and Artemia. Nevertheless, both demonstrated good characteristics as probiotic candidates for aquaculture.

ABSTRAK

Probiotik adalah bacteria yang boleh bertindak sebagai penggalak kesihatan organisma lain dengan mengubah masyarakat mikrob ambient, meningkatkan nilai pemakanan makanan, meningkatkan tindak balas perumah ke arah penyakit, atau meningkatkan kualiti alam sekitar di sekelilingnya. Probiotik *Micrococcus* spp. (JAQ07) dan *Bacillus* spp. (JAQ04) telah digunakan sebagai probiotik yang berpotensi dalam eksperimen ini. Kedua-dua probiotik telah dikenal pasti sebagai Gram-positif dengan morfologi yang berbeza. *Micrococcus* spp. adalah bacteria berbentuk rod manakala *Bacillus* spp. adalah bacteria berbentuk cocci. Dalam pengujian *in-vivo*, Artemia telah digunakan sebagai perumah dan dirawat dengan *Bacillus* spp. dan *Micrococcus* spp. pada kepekatan yang berbeza (10^2, 10^4 and 10^6 CFU ml^{-1}) dan kemudian dicabar dengan *Vibrio alginolyticus* pada kepekatan 10^5 CFU ml^{-1}. *Bacillus* spp. dapat meningkatkan hidup Artemia lebih baik berbanding dengan *Micrococcus* spp. apabila dicabar dengan *Vibrio alginolyticus*. Artemia yang dirawat dengan *Bacillus* spp. dan dicabar dengan *Vibrio alginolyticus* menunjukkan 70% kadar hidup berbanding dengan kadar hidup Artemia yang dicabar dengan *Vibrio alginolyticus* sahaja (20% kadar hidup) selepas 7 hari. Sementara itu, pra-inkubasi Artemia dengan *Micrococcuc* spp. dan dicabar dengan *Vibrio alginolyticus* menunjukkan 68% kadar hidup. Kedua-dua probiotik tidak berbahaya kerana kadar hidup yang tidak signifikan telah ditemui berbanding dengan kawalan. *Micrococcus* spp. berupaya untuk mengurangkan sedikit beban vibrio dalam Artemia dan kultur air. Walau bagaimana, *Bacillus* spp. tidak mampu untuk mengurangkan beban vibrio dalam kultur air dan Artemia. Walau bagaimanapun, kedua-duanya menunjukkan ciri-ciri baik sebagai calon probiotik untuk akuakultur.

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LIST OF ABBREVIATIONS/ SYMBOLS

JAQ07	*Micrococcus* spp.
JAQ04	*Bacillus* spp.
VA	*Vibrio alginolyticus*
TSA	Trypicase Soy Agar
TSB	Trypicase Soy Broth
TCBS	Thiosulfate Citrate Bile Salts Sucrose
NaCl	Sodium Chloride
rpm	round per minute
ppt	part per thousand
ml	milliliter
µL	microliter
µm	micrometer
mm	milimeter
g	gram
ssw	sterile sea water
°C	degree centrigrade
%	percent
CFU	colony forming unit
CFU ml^{-1}	colony forming unit per mililiter
mgL^{-1}	milligram per liter
DO	Dissolved oxygen
NH_3-N	Ammonia nitrogen

CHAPTER 1

INTRODUCTION

The demand for aquaculture product is increasing throughout the year because of the increasing of population growth and the increasing rate of consumption on aquaculture product. According to Food and Agriculture Organization of the United Nations (FAO), the aquaculture product is increasing from 78 091 908 tones in 2010 to 83 729 313 tones in 2011. However, due to the intensive culture aquaculture diseases outbreak has occurred. Diseases outbreak are caused by several factors such as high stoking density, bad water management and feeding cultured fish with bad feed. These conditions had lead to infection to cultured fish by viruses, parasites, fungi and bacteria.

Vibriosis is a bacterial fish disease that caused by *Vibrio* species which mainly affect marine fish species and caused many losses in marine fish culture. The general symptoms for this disease are haemorrhages and superficial skin lesion and general septicemia in most cases. Colwell and Grimes (1984) had listed eight *Vibrio* species as fish pathogens in marine fish populations. The most common pathogenic *Vibrio* species are *Vibrio cholerae* (Muroga *et al*., 1979; Yamanoi *et al*., 1980), *Vibrio vulnificus* (Tison *et al*., 1982), *Vibrio ordalii* (Schiewe *et al*., 1981), *Vibrio damsela* (Love *et al*., 1981) and *Vibrio salmonicida* (Egidius *et al*., 1986; Wiik and Egidius, 1986).

There are many ways that can be used to overcome bacterial diseases such as administration of antibiotics into the water (Brown, 1989). However, since the use of antibiotics can promote the development of antibiotic-resistant bacteria in fish and also the environment (Alderman and Hastings, 1998; Brown, 1989; Pedersen *et al*., 1995; Smith *et al*., 1994; Hameed and Balasubramanian, 2000; Bjorklund *et al*., 1991), introduction of bacterial probiotics such as lactic acid bacteria (Villamil *et* al., 2003; Verschuere *et al*., 2000; Balcázar *et al*., 2008; Ringø and Gatesoupe, 1998; Gatesoupe, 1991, 1994, 1999) can be used as an alternative solutions.

Probiotics are bacteria that promote the health of other organisms (Balcázar *et al*., 2006). Irianto and Austin (2002a) stated that "a probiotic is an entire or components(s) of a microorganism that is beneficial to the health of the host". The mechanisms of the probiotics include pathogen inhibition through production of antagonistic compounds, competition for essential nutrients and attachment sites, alteration of enzymatic activity leads to development of immunity response, feed digestibility and utilization and also modulation of interactions with the environments (Gomez and Balcázar, 2008; Bomba *et al*., 2002; Verschuere *et* al., 2000; Fooks *et* al., 1999; Ringø and Gatesoupe, 1998; Fuller, 1989).

Artemia has been widely used as live feed for marine finfishes and crustacean hatcheries (Lavens and Sorgeloos, 1986). They have been recognized as the best larval food source because of the unique production of cysts. Besides that, Artemia can also be consumed directly by decapsulating the cysts with hypochlorite solution or right after the

incubation period. Artemia has been used as a vector for probiotics delivery agents (Patra and Mohamed, 2003; Gatesoupe, 1994) and thus, the objectives of this study are:

i. To determine the ability of probiotics to enhance the survival of Artemia.

ii. To determine the ability of probiotics to reduce the number of pathogenic *Vibrio* in Artemia culture.

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