UNIVERSITI PUTRA MALAYSIA

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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:

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ABSTRACT

Bacterial bioremediators were isolated from microalgae and grouper culture water. Three potential bioremediators were isolated each from microalgae *Chlorella vulgaris* and grouper culture water. The total ammonia degradation test showed that all strains degraded ammonia at 0.5 ppm. The strain BP-GRP/2 was further tested to grouper juveniles culture. Although, no significant differences can be seen on TAN and SRP, nitrite level was decreased in the presence of the strain. The fish also survived (100% survival) well compared to control (67% survival) in eight days of experiment. The strain was a Gram-negative bacterium by using Gram staining method and molecularly identified as *Aeromonas hydrophila* by 16S rRNA gene sequencing. These results showed that certain bacterial strain can act as bioremediator and improve the survival of the host.

ABSTRAK

Bakteria bioremediasi telah dipencil daripada air ternakan mikroalga dan ikan kerapu. Tiga bioremediator berpotensi masing-masing terpencil daripada air ternakan *Chlorella vulgaris* dan ikan kerapu. Jumlah ujian degradasi ammonia menunjukkan bahawa semua strain menurunkan ammonia pada 0.5 ppm. Strain BP-GRP/2 telah diuji keatas ternakan ikan kerapu juvana. Walaupun, tiada perbezaan yang signifikan dapat dilihat pada TAN dan SRP, tahap nitrit telah berkurangan dengan kewujudan strain itu. Ikan juga hidup (100%) berbanding dengan kawalan (67%) semasa lapan hari eksperimen. Strain itu ialah bakteria Gram-negatif dengan mengunakan kaedah pewarnaan Gram dan dikenalpasti secara molekul sebagai *Aeromonas hydrophila* oleh penjujukan gen 16S rRNA. Keputusan ini menunjukkan bahawa strain bakteria tertentu boleh bertindak sebagai bioremediator dan meningkatkan hidup tuan rumah.

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LIST OF ABBREVIATIONS/ SYMBOLS

(NH4)2SO4	Ammonia Sulphate
Na2[Fe(CN)5NO]2H2O	Sodium Nitroprusside
NO2-N	Nitrite
$MgCl_2$	Magnesium Chloride
DDH2O	Double Distilled Water
dNTPs	Deoxynucleotide Triphosphates
PCR	Polymerase Chain Reaction
TAN	Total Ammonia-Nitrogen
SRP	Soluble Reactive Phosphorus
EUS	Epizootic Ulcerative Syndrome
CFU/ml	Colony Forming Unit per ml
DO	Dissolved Oxygen
rpm	Revolutions per minute
ppm	Parts per million
ml	Milliliter
U/ml	Unit per milliliter
mM	Millimolar
mm	Milimetre
µl	Microliter
µg	Microgram

g	Gram
µm	Micrometre
nm	Nanometre
%	Percent
°C	Degree centrigrade
v/v	volume per volume

CHAPTER 1

INTRODUCTION

Nowadays, beneficial bacteria are commonly used for many purposes due to the demand for environmentally friendly and economically profitable aquaculture. Beneficial bacteria known as probiotics are needed against pathogen and pollutions. Bacterial bioremediators is valuable in aquaculture due to its role in mass transfer and utilization of substrate for water quality improvement in aquaculture (Wang and Han, 2007). According to Sharma and Reddy (2004), bioremediation is a process that completely breakdown the pollutants into non-toxic compound where this process does not involve transferring of contaminants to other another environmental medium. Bacterial bioremediators isolated from microalgae are seen more capable because bacteria and microalgae are significantly related in aquaculture and can be used together in consortium.

Meanwhile, grouper culture (*Epinephelus* sp.) is widely found in the coastal water of Malaysia and was first introduced in 1973 in net cages (FAO, 1991). It is a high value fish due to the demand of it. Fish farmer in Malaysia have been importing large numbers of hatchery-produced fish, *E. lanceolatus*, *E. fuscoguttatas* and *Cromileptes altivelis* from Taiwan in the past few years. The fishes are also susceptible to diseases (Pomeroy, 2007). According to Wong and Leong (1987), in years 1989, Malaysia was estimated to loss US$ 1.3 million in potential income combined with private sector and government farms due to diseases of cage cultured grouper. Poor water quality is the main factor of disease infections.

Bacterial bioremediators might be the solution to improve water quality in grouper culture. Excess ammonia from the grouper feeding and wastage can be controlled in the presence of bioremediators. Fish exposed to high levels of ammonia over time are more susceptible to bacterial infections and causes stress and gill damage, poor growth and will not tolerate routine handling (Floyd and Watson, 2006). In other perspective, Vidali (2001) reported that bioremediation uses relatively low-cost, low-technology technique, which generally have a high public acceptance and can often be carried out on site. This can be done through bioremediation using living organisms (bacteria, fungi, actinomycetes, cyanobacteria, microalgae and to a lesser extent, plants) to reduce the concentration or toxicity of a pollutant which microorganisms can treat in the wastewater.

Thus the objectives of this study are:

i. To isolate bacteria with bioremediation properties from microalgae and grouper culture water,

ii. to determine the effects of the bacterial bioremediator on the survival and water quality of grouper culture.

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