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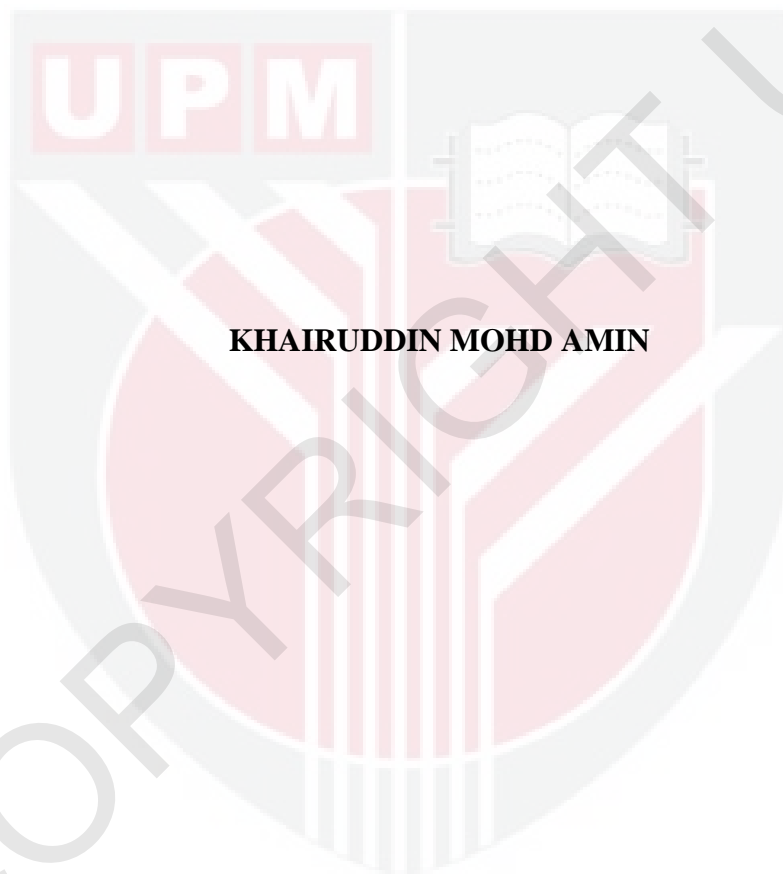
***ISOLATION OF MARINE BACTERIA WITH BIOREMEDIATION
PROPERTIES FROM THE CULTURE WATER OF TIGER GROUPER
(*Epinephelus Fuscoguttatus*)***

KHAIRUDDIN MOHD AMIN

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153763

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

**DEPARTMENT OF AQUACULTURE,
FACULTY OF AGRICULTURE,
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2012

CERTIFICATION OF APPROVAL
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properties from the culture water of Tiger grouper
(*Epinephelus fuscoguttatus*)

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 AKU 4999 project guideline, Department of Aquaculture, Faculty of Agriculture, University Putra Malaysia.

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ABSTRACT

An experiment was conducted to isolate bacterial bioremediator from the culture water of Tiger Grouper (*Epinephelus fuscoguttatus*). In this study, the potential of the bacteria as ammonia degraders were investigated. This was done through enrichment assay using ammonium sulphate as carbon source. The bacteria were grown in minimal medium and sodium chloride. Through this assay, three distinct bacteria were isolated. Using the total ammonia-nitrogen (TAN) analysis, all three bacteria were able to degrade ammonium sulphate up to 0.04 ppm compared to the control of 0.54 ppm. All three bacteria were found to be Gram-positive bacteria by its distinctive purple color in Gram-staining method. Based on result, it can be concluded that the marine bacteria isolated from the culture water of Tiger grouper have the potential as bioremediator agent in aquaculture.

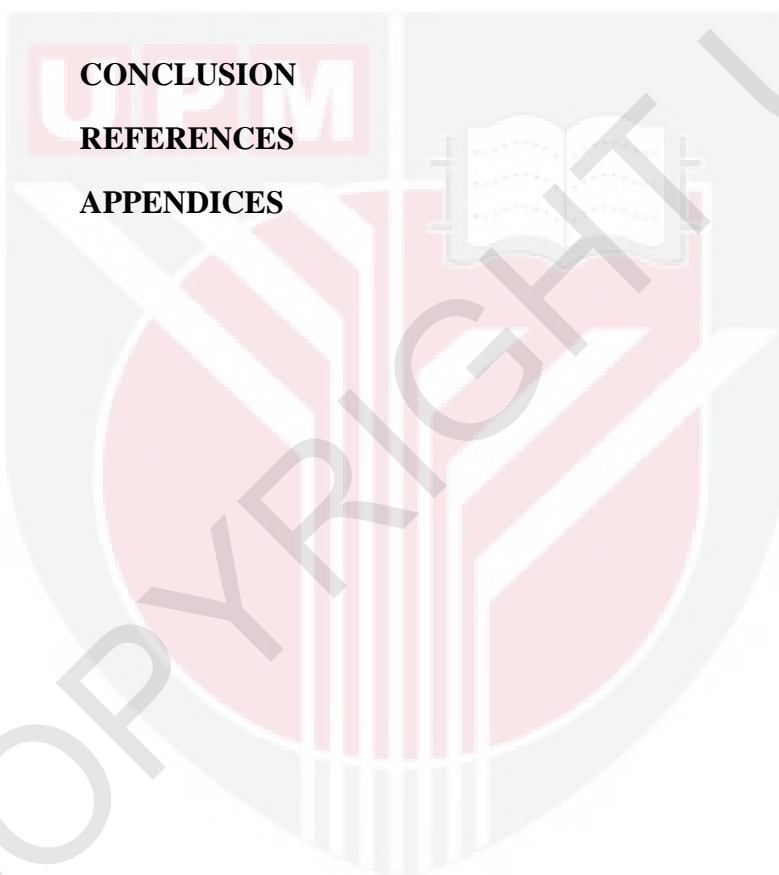
ABSTRAK

Satu kajian telah dijalankan untuk memencilkan “*bioremediator*” bakteria daripada kultur air kerapu harimau (*Epinephelus fuscoguttatus*). Dalam kajian ini, potensi bakteria sebagai penghurai ammonia telah dikaji. Kajian telah dilakukan dengan menggunakan ammonium sulfat sebagai sumber karbon. Bakteria mengalami pertumbuhan di dalam minimum media dan sodium klorida. Melalui teknik cerakin ini, tiga bakteria yang berbeza telah dipencilkan. Dengan menggunakan analisis jumlah ammonia-nitrogen (TAN), ketiga-tiga bakteria dapat merendahkan ammonium sulfat sehingga 0.04 ppm berbanding kawalan 0.54 ppm. Ketiga-tiga bakteria ini didapati adalah bakteria Gram-positif kerana ianya berwarna ungu melalui kaedah ‘*Gram-staining*’. Berdasarkan keputusan, kesimpulan diperolehi bahawa marin bakteria yang diasingkan daripada kultur air kerapu harimau mempunyai potensi sebagai agen “*bioremediator*” di dalam akuakultur.

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LIST OF ABBREVIATIONS / SYMBOLS

NaCl	Sodium Chloride
$(\text{NH}_4)_2\text{SO}_4$	Ammonium Sulphate
CO_2	Carbon Dioxide
DDH ₂ O	Double Distilled water
H ₂ O	Water
KH_2PO_4	Monopotassium Phosphate
MgSO_4	Magnesium Sulphate
$\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]_2\text{H}_2\text{O}$	Sodium Nitroprusside, dihydrate
Na_3PO_4	Sodium Phosphate
NH_4Cl	Ammonium Chloride
TAN	Total Ammonia-Nitrogen

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CHAPTER 1

INTRODUCTION

Epinephelus fuscoguttatus or Tiger Grouper are the most popular carnivorous fish with high market value in the world such as Kuwait, Indonesia, USA, Japan, Mexico, Taiwan, China, Philippines, Thailand and Malaysia. Global grouper production increased in recent years with 60 774 mt, 99 378 mt, 163 093 mt and 198, 690 mt in 1990, 2000, 2005, and 2007, respectively (FAO, 2005a,b; 2009). Brown-marbled grouper become an important mari-culture fish for intensive aquaculture, particularly in the Asia Pacific region because of their high consumer demand, desirable taste, hardiness in a crowd environment, fast growth, efficiency in feed conversion and rapid growth (Sim *et al.*, 2005; Chen and Tsai, 1994; Kohno *et al.*, 1993). Wild grouper culture become emerging as a viable venture besides from overexploitation in many countries. Because of the importance value as a cultured fish, grouper have become a challenging target research for aquaculture scientists (Kohno *et al.*, 1993).

Tiger Groupers are high priced and popular seafood fishes in Southeast Asia (Sarjito *et al.*, 2009). *Epinephelus* sp. is an aquaculture species that are widely used for intensive farming in world (Boonyaratpalin, 1997; Haemstra and Randall, 1993). However this intensive culture of grouper has been severely hit by viruses and bacteria, which have caused serious economic losses (Chiu *et al.*, 2008) both in farms and hatcheries. Intracohort cannibalism usually results in mass mortality, during larviculture (Fukuda *et al.*, 1999 ; Watanabe *et al.*, 1991). Tiger Grouper

farming has suffered several disease problem like nervous necrosis and sleepy disease as well as vibriosis caused by *Vibrio alginolyticus* (Fukuda *et al.*, 1993). Palanisamy *et al.* (1999) reported that grouper farm or hatcheries at Malaysia, commonly faces vibriosis disease caused by *Vibrio* spp.

Aquaculture has been supporting human demands for fish consumptions for a centuries and is an important industry around the world (Chopin and Yarish, 1998). Intensive scale fish aquaculture farming has caused many environmental problems to farmer (Wu, 1995). Usually modern intensive monoculture farm requires huge input of water, feeds, fertilizers and chemicals but produces a lot of wastes. Waste products from fish farms constitutes of nitrogen, phosphorus and carbon dioxide (Chung *et al.*, 2002). Many studies on biological nitrogen removal through nitrification, denitrification and anaerobic ammonium oxidation (annamox) have been done and many bacterial group have been described (Crooker and Contreras, 2010). But a lot more efforts must be put on bioremediation of aquaculture waste water which allow more environmental balanced aquaculture practices.

Therefore the objective of this study is :

1. To isolate bacterial bioremediator from the culture water of Tiger Grouper

(*Epinephelus fuscoguttatus*)



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