UNIVERSITI PUTRA MALAYSIA

INTERNAL TRANSCRIBED REGION (ITS) GENE SEQUENCING AND PATHOGENICITY STUDY OF Aeromonas veronii bv. sobriaSTRAIN KTG3SB IN JUVENILE RED TILAPIA.

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

DEPARTMENT OF AQUACULTURE

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CERTIFICATION OF APPROVAL

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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.

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ABSTRACT

The present study was conducted to identify the *Aeromonas* spp.by using Internal Transcribed Region (ITS) gene sequencing and to determine the virulence of the species. Five samples labelled TPK4B, PHE578L, KTG3SB, TPK2B and PK159S isolated from diseased freshwater fishes. The API test of all samples shows that the species were*A. sobria*. In contrast, ITS sequence of three samples; TPK4B, PHE578L and KTG3SB were identified as *A. veronii bv. sobria* with similarity 97-98%. Another two samples TPK2B and PK159S were identified as *A. culicicola* and *A. hydrophila* respectively. Virulence of sample KTG3SB to juvenile red tilapia was also tested. 150 fish were injected through IP injection with different bacterial concentration ($0.1x10^3$ – $0.1x10^7$ CFU/ml). Other 30 fish were injected with sterile PBS as negative control. The mortality recorded every two hours post challenge for three days. The LD_{50} calculated was 10^3 CFU/ml of treatment groups and fish shows symptom such as congestion, enlargement of gall bladder and abnormal swimming. The histology finding also shows some congestion in liver, spleen and gill. In the present study, ITS gene sequencing had proved that this method can definitely distinguish between intraspecific species and *A. veronii bv. sobria* had proved that it is very pathogenic to juvenile red tilapia.

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ABSTRAK

Kajian ini telah dijalankan untuk mengenal pasti *Aeromonas* spp. dengan menggunakan penyusunan gen Internal Transcribed Region (ITS) dan untuk menentukan kadar kebisaan sepsis tersebut. Lima sampel yang dilabel TPK4B , PHE578L , KTG3SB , TPK2B dan PK159S diambil dari ikan yang telah dijangkiti penyakit. Ujian API bagi semua sampel menunjukkan bahawa spesiestersebut adalah*A. sobria* . Sebaliknya, menurut ITS, tiga sampel; TPK4B, PHE578L dan KTG3SB telah dikenalpasti sebagai *A. veronii bv. sobria* dengan nilai persamaan 97-98%. Sample yang lain iaitu TPK2B dan PK159S telah dikenalpasti sebagai *A. culicicola* dan *A. hydrophila* setiap satu.Kebisaan sampel KTG3SB bagi juvana tilapia merah telah juga diuji. 150 ikan telah disuntik melalui suntikan IP dengan kepekatan bakteria yang berbeza (0.1x103 - 0.1x107 CFU/ml). 30 ikan yang selebihnya telah disuntik dengan steril PBS sebagaikawalan negatif. Kematian direkodkan setiap dua jam selama tiga hari. LD_{50} dikira adalah 10^3 CFU/ml dan ikan menunjukkan simptom seperti pendarahan, pembesaran pundi hempedu dan berenang yang tidak normal. Penemuan histologi juga menunjukkan pendarahan di dalam hati , limpa dan insang dalam kumpulan rawatan. Dalam kajian ini, penyusunan gen ITS telah membuktikan bahawa kaedah ini boleh membezakan antara spesies intraspesies dan *A. veronii bv . sobria* telah membuktikan bahawa ia adalah sangat berbisa kepada juvana tilapia merah.

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LIST OF ABBREVATION AND SYMBOLS

α	alpha
β	beta
^{o}C	Degree centrigade
µL	microliter
bp	base pair
g	gram
min	minutes
mm	millimetre
mM	micromolar
ml	millilitre
rpm	revolutions per minute
sec	second
LD_{50}	Lethal Dose 50%
API TEST	Analytical Profile Index test
BLAST	Basic Local Aligment Search Tool

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CFU	Colony Forming Unit
EDTA	Ethylenediaminetetraacetic acid
GDP	Gross Domestic Product
RAPD	Random Amplified Polymorphism DNA

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CHAPTER 1

INTRODUCTION

Tilapia or *Oreochromis* spp. are the most commonly cultured species in many farms around the world. Like other fish species, tilapia also was very susceptible to infectious diseases that have been caused by parasite, viruses as well as bacteria. All this kind of pathogen were very harmful and can caused heavy mortality of the cultured species. The most popular disease of tilapia caused by bacteria is Streptococcosis caused by *Streptococcus* spp. and Motile Aeromonad Septicemia (MAS) caused by *Aeromonas* spp. Problem that related with *Aeromonas* spp. is they can cause 'fin rot' or 'skin rot' diseases that lead to heavy mortality in cultured tilapia (El Sayed, 2006). *Aeromonas* are the main cause of bacterial infections affecting tilapia, *Oreochromis niloticus* (Li and Cai, 2011). For example, *Aeromonas veronii bv. sobria*which is the causal agent of Epizootic Ulcerative Syndrome (EUS) of fish in Bangladesh (Rahman *et al.,*2002). The other diseases caused by *Aeromonas* spp. are furuncolosis caused by *Aeromonas salmonicida* that affected most cultured salmonid fishes (O' Brien *et al.,*1994) and caudal fin rot diseases caused by *Aeromonas hydrophila* (El Sayed, 2006).

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To manage this problem, antimicrobial agents are always tested with *Aeromonas* spp. (Ghenghesh *et al.,* 2008). Research has found that *Aeromonas* spp. was highly resistance to antimicrobial agent such as ampicilin, chloramphenicol and enroflocaxin and sensitive to antimicrobial agent such as ciproflocaxin, tetracycline and erythromycin (Li and Cai, 2011). The studies on diseases development of fish species are important so that the treatment and preventive measures of the diseases can be determined by studying the specific causal agent of diseases development.

In this present day, the use of Intergenic Transcribed Spacer (ITS) region gene sequencing has been the most common method to study the bacterial taxonomy compared to the other method of bacterial identification such as 16S rRNA and RAPD. This is mainly because these regions have greater variability compared to adjacent genes (Daffonchio *et al*., 2003). Thus, molecular identification of the pathogen that causes the diseases is important so that further research on the pathogen as well as the ways to handle it may be established. This is due to molecular identification has more advantages over conventional methods of microscopic examination. Identification of sample can be determined using only small amount of material, and it is more accurate than with previous conventional method *(*Vossbrinck, 1991).

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Although the study of the virulence factor of aeromonad species had been done before, the pathogenesis as well as the virulent factor for chosen *Aeromonas* spp. infection are not well be understood (Rahman *et al*., 2002). Thus, more research need to be done on *Aeromonas* species especially *Aeromonas veronii bv. sobria* and its virulence properties. In this case, these studies were established to address the following objectives:

i) To identify five isolates of *Aeromonas* spp. isolated from diseased cultured fish by using Internal Transcribed Spacer (ITS) Gene Sequencing.

ii) To determine the virulence of the pathogenic agent *Aeromonas veronii bv. sobria*strain KTG3SBin juvenile red tilapia.

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APPENDICES