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MOLECULAR CHARACTERIZATION AND ANTAGONISM ASSESMENT OF BACTERIAL CANDIDATES AS PROBIOTICS FOR FISH AQUACULTURE

MOHAMAD PIPUDIN BIN ABDUL AZIZ

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MOHAMAD PIPUDIN BIN ABDUL AZIZ
158412

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

DEPARTMENT OF AQUACULTURE
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CERTIFICATION OF APPROVAL

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FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

Name of student : Mohamad Pipudin Bin Abdul Aziz

Matric number : 158412

Programme : Bachelor of Agriculture (Aquaculture)

Year : 2013

Name of supervisor : Dr. Ina Salwany bt. Md. Yasin

Title of Project : Molecular Characterization and Antagonism Assesment of Bacterial Candidates as Probiotics for Fish Aquaculture

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/& co-supervisor:

_____

Supervisor's name

Date:

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ABSTRACT

The antagonistic ability of candidate probiotics *Bacillus* JAQ 04 and *Micrococcus* JAQ 07 towards selected fish pathogen *Vibrio alginolyticus* ATCC33839 were conducted in liquids modes via co-culture assay. Each probiotics were prepare in 3 different concentrations (10^2, 10^4 and 10^6 CFU ml^{-1}) and each concentration inoculated with pathogen *Vibrio alginolyticus* 10^5 CFU ml^{-1} in liquid media of Tryptic Soy Broth to allow the antagonistic activity for 120 hours, the effectiveness of antagonistic will be measure by the reduction of *V. alginolyticus* colonies via plating count for each 24 hours interval for 120 hours. The observation of antagonistic effect of co-culture assays shows the reduction of *V. alginolyticus* colonies were reduced successfully by both probiotics strains compare to the control (*V. alginolyticus* 10^5 $CFUml^{-1}$ alone). Bacillus strain JAQ 04 with high concentration ($10^6 CFUml^{-1}$) showed the effectiveness antagonism compare to Micrococcus strain JAQ 07. Bacillus strain JAQ 04 then assessed for molecular study. Studies in molecular level using PCR "Internal Transcribed Spacer (ITS)" is important to ensure that Bacillus strain JAQ 04 as a safety

ABSTRAK

Keupayaan antagonistik probiotik antara *Bacillus* strain JAQ 04 dan *Micrococcus* strain JAQ 07 terhadap patogen ikan yang dipilih iaitu *Vibrio alginolyticus* ATCC 33839. Kajian dijalankan pada mod cecair melalui kaedah pencampuran antara probiotik dan patogen dlm satu medium cecair. Setiap probiotik telah disediakan dalam tiga kepekatan yang berbeza (10^2,10^4 dan 10^6 CFU ml^{-1}). Setiap kepekatan akan disuntik dengan patogen *Vibrio alginolyticus* berkepekatan 10^5 CFU ml^{-1} dalam media cecair Tryptic Soya Broth (TSB) untuk membenarkan aktiviti antagonistik antara patogen dan probiotik selama 120 jam. Keberkesanan antagonistik akan diukur melalui pengurangan koloni *V. alginolyticus* melalui kaedah pengiraan plat, kiraan dilakukan bagi setiap selang 24 jam selama 120 jam. Pemerhatian terhadap antagonistik di dalam kaedah pencampuran probiotik dan patogen di dalam cecair menunjukkan bahawa koloni *V. alginolyticus* telah berkurangan dengan jayanya jika dibandingkan dengan kumpulan kawalan (*V. alginolyticus* 10^5 CFU ml^{-1} sahaja). *Bacillus* strain JAQ 04 menunjukkan keberkesanan dan kepekatan yang paling terbaik iaitu 10^6 CFU ml^{-1} berbanding *Micrococcus* strain JAQ 07 Kajian diperingkat molekul menggunakan PCR "Internal Transcribed Space (ITS)"adalah penting bagi memastikan bahawa strain JAQ 04 dan JAQ 07 adalah produk probiotik yang selamat untuk kajian yang akan datang dan juga aplikasi penggunaannya di dalam sistem akuakultur.

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LIST OF ABBREVIATIONS/SYMBOLS

Abbreviations/Symbols

%	Percent
&	And
µl	Microlitres
^{0}C	Degree Celsius
CFU ml^{-1}	Colonies forming unit per milliliter
Fig.	Figure
nm	Nanometer
G	Gram
Ml	Milliliter
No.	Numbers
Sp	Species
™	Trademark
TSA	Tryptic™ soy agar
TSB	Tryptic™ soy broth
TCBS	Thiosulfate-Citrate-Bile-Sucrose Agar
USA	United States of America

CHAPTER 1

INTRODUCTION

Aquaculture is one of the main industries that are fast-growing and rapidly expanding to fulfill the demand of their aquaculture product where the main product of aquaculture is supporting food consumption of human. In the mean time, the health of farmed fish is significantly affected by the microbial pathologies and other bacterial in origin during the aquaculture activity. FAO (2012) report that the state of the world fisheries and aquaculture indicates in 2011 a global aquaculture production of 63.6 million tons (aquatic plants excluded) and global marine catches of 90 million tones. Around 27.3 million tones of global marine catches were destined for non-food purpose, of which 20 million tones went into reduction to fishmeal and fish oil. Over the last decade, the data accumulation from FAO showed the world aquaculture production continuously growing, while during the same period the amount of captured fish used for fishmeal and fish oil production remained relatively stable.

Since the production of the aquaculture is a food base product the serious concern of this industry is the safety of the product which includes the surround environment of the aquaculture production, health of product, use of chemicals and treatments and disease constrain method. Early solution of treatment towards the disease control of fish is by using antibiotics, the use of antibiotics to control disease was widely practiced and unfortunately indiscriminate used of this

chemotherapeutic agent led to emergence of numerous antibiotic-resistant bacteria, as a result more dosage of antibiotics to overcome and counter the pathogens resistant and become an issue of food security and safety such as the risk of transferring the antibiotic-resistance plasmid to human pathogenic bacteria. Thereby the aquaculture production crashed in many Asian countries which seriously involve in practicing antibiotics in aquaculture production (Karunasagar *et al.,* 1994). As a result, currently, antibiotics are no longer effective in dealing with aquaculture environment disease (Defoirdt *et al*., 2007) and the utilization of antibiotics were implemented bans on by European Union and USA to be practice in aquaculture (Kesarcodi-Watson *et al.,* 2008) Several methods have been develop since the bans of antibiotics usage such as filtration of water, addition of sodium chloride, ozonation, ultraviolet light treatment, etc those methods are able to overcome the disease but not as effective as probiotics (Kesarcodi-Watson *et al.,* 2008). Probiotics were admittedly as potential alternative among other alternatives, several study support the benefits beyond the practicing of probiotics. Probiotics have been defined as "live microbial feed supplement that can benefit the host by improving its intestinal balance" (Fuller, 1989). As living microorganisms, they produce no drugs resistance, excrete harmful chemicals and drug residues which can effect and transferring to the consumers (Scharek *et al*., 2005).

Probiotics role as microbial intervention which is vital in production and growth of aquaculture, effective treatments may provide broad spectrum and greater nonspecific disease protection (Panigrahi and Azad, 2007). The range of probiotics micro-organisms examined for used in aquaculture includes both Gram-

negative and Gram-positive bacteria, bactiriophages, yeast and unicellular algae (Irianto and Austin, 2002). The selection of probiotics candidate was based on in vitro antagonism ability (Vercshuere *et al.*, 2000), as well as on the result of adhesions, colonization and growth in intestinal mucus (Vine *et al.*, 2006). Conventional culture-based techniques, in vitro or in vivo methods and others methods with even in several different media have been implemented to test the antagonism ability of candidate probiotics for their selection it does not present a correct picture of bacterial diversity. Therefore, to present more reliable information about the probiotics selected, molecular methods are used. One of the most popular used methods in probiotics is Polymerase Chain Reaction-Denaturating Gradient Gel Electrophoresis (PCR-DGGE), as the method is reliable, rapid, sensitive and easy to use to study microbial diversity (Zhou and Liu, 2008).

The purpose of this study was therefore:

1) To emphasize the evaluation of antagonism ability of candidates probiotics of *Bacillus* JAQ 04 and *Micrococcus* JAQ 07 to inhibit pathogenic bacteria of *Vibrio alginolyticus.*

2) To characterize selected probiont by molecular Internal Transcribe Spacer (ITS) region gene sequencing related on the organism safety.

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