SCREENING OF POTENTIAL DIATOMS FROM SHRIMP POND WITH QUORUM QUENCHING PROPERTIES

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This project report is submitted in partial fulfillment of the requirements for the 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 year 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 Aqauculture, Faculty of Agriculture, Universiti Putra Malaysia.

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ABSTRACT

Quorum sensing is a mechanism which bacterial cell to cell communication that regulates the expression of various genes including virulence genes. In this study, the samples of diatoms are Strain BPD1, *Amphora* sp., *Halamphora* sp., *Chaetoceros* sp. and *Thalassiosira* sp. The quorum quenching properties from different diatom species were screened using reporter strain *Chromobacterium violaceum* CV026. The diameter of the inhibition zone were measured to identify the most active quorum quenching diatom. The unidentified diatom was extracted using 18S rRNA analysis and identified as *Denticula* sp. From 2 out of 5 diatoms, *Denticula* sp. and *Amphora* sp. showing as active quorum quenchers. The most active is *Denticula* sp. as it can inhibit the reporter strain CV026 in lower concentrations compare to *Amphora* sp. In this study, it showed that the diatoms with quorum quenching properties can be used as disease control in shrimp aquaculture.

Keywords: quorum quenching, diatom

ABSTRAK

Kuorum penderiaan adalah mekanisme bakteria diamana sel berkomunikasi antara satu sama lain untuk mengatur ekspresi berbagai gen termasuk gen virulensi. Dalam kajian ini, sampel diatom adalah Strain BPD1, *Amphora* sp., *Halamphora* sp., *Chaetoceros* sp. dan *Thalassiosira* sp. Ciri anti-kuorum penderiaan dari spesies diatom yang berbeza telah ditayangkan menggunakan pelapor strain *Chromobacterium violaceum* CV026. Diameter zon inhibisi diukur untuk mengenalpasti diatom yang paling aktif dalam aktiviti anti-kuorum penderiaan. Strain BPD1 telah diekstrak menggunakan analisis 18S rRNA dan dikenali sebagai *Denticula* sp. Dua daripada lima diatom, *Denticula* sp. dan *Amphora* sp. telah menunjukkan aktiviti anti-kuorum penderiaan. *Denticula* sp. adalah paling aktif dalam aktiviti anti-kuorum penderiaan kerana ia boleh menghalang pelapor strain CV026 dalam kepekatan yang lebih rendah berbanding dengan *Amphora* sp. Dalam kajian ini, ia menunjukkan bahawa diatom dengan sifat anti-kuorum penderiaan boleh digunakan sebagai kawalan penyakit dalam penternakan akuakultur udang.

Kata kunci: kuorum penderiaan, diatom

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LIST OF ABBREVIATIONS AND SYMBOLS

rpm	revolutions per minute
LB	Luria Bertani
^{o}C	degree centrigade
ml	milliliter
µl	microliter
mm	millimeter
ppm	parts per million
nm	nanometers
QS	Quorum sensing
QQ	Quorum quenching
CFU	colony forming unit
AHL	Acyl Homoserine Lactones
DNA	Deoxyribonucleic acid
PCR	Polymerase Chain Reaction
Mg^{2+}	Magnesium Chloride
dNTPs	Deoxynucleotide Triphosphate
EDTA	Ethylenediaminetetracetic acid
TBE	Tric Borate EDTA
bp	Base pair

CHAPTER 1

INTRODUCTION

Microalgae are microscopic plants without roots or leaves with diameter between 1-50 micrometers. Microalgae are very common and they exist in freshwater and seawater where mainly become the basis of food chains. Most species of microalgae contain chlorophyll which use sunlight as and energy source and convert carbon dioxide (Wolkers, 2011). According to Metting (1996), microalgae are great in diversity and are important in many fields such as health foods, aquaculture feeds, waste treatment and biofertilizers. In aquaculture, microalgae are often used as added nutrition and as component to basic nutrients (Hemaiswarya et al., 2011; Brown and Blackburn, 2013). Microalgae are important during the larval stages of aquatic species, either as direct feeding for molluscs and shrimps or indirect food for live prey fed to the small fish larvae e.g., rotifer and *Artemia* (Muller, 2000). Among the microalgae that are commonly used in aquaculture consist of *Chlorella*, *Tetraselmis*, *Scenedesmus*, *Pavlova*, *Phaedoctylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema* and *Thalassiosira*. These species of microalgae have high growth rate and tolerance in different temperature, light and nutrients (Sirakov et al., 2015).

Diatoms are unicellular and eukaryotic microscopic organisms which classified in Kingdom Protista. The characteristic of diatoms is the cell wall which en masse heavily impregnated with silica.

Besides that, diatoms can also generate wide range of chemical compounds that are associated with many types of bioactivity such as antibacterial activity against pathogenic bacteria (Molina and del Pilar, 2017). Diatoms are one of the beneficial algae in aquaculture due to their biochemical compositions and can be well fed and digested by aquatic animals.

To date, aquaculture is one of the fastest growing field for food productions. However, disease problems are increasing as aquaculture growth intensifies and are among the main restrictions in the culture of many aquatic species (Bondad-Reantaso et al., 2005). In aquaculture, pathogens such as *Aeromonas* sp., *Vibrio* sp. and *Pseudomonas* sp. affect aquatic organisms causing high mortality in aquatic animal (Defoirdt et al., 2004; Lafferty et al., 2015). Antibiotics have been used to control the pathogens. However, overuse of antibiotic can lead to antibiotic resistant towards the pathogens (Defoirdt et al., 2011).

A number of studies revealed that one of the possible techniques for disease control is through disruption of quorum sensing (QS) or bacterial cell-to-cell signalling. According to Natrah et al. (2011), quorum sensing showed to regulate the virulence of various aquaculture pathogens. Interference of bacterial QS signal molecules by different organisms such as bacteria, microalgae have been reported (Defoirdt et al., 2004). Hence, quorum sensing interference by diatoms have the potential to be used in preventing bacterial disease in aquaculture.

The objectives of this study are:

1. To screen quorum quenchers from different species of diatoms.
2. To identify the selected and potential diatom strain using 18S rRNA gene sequencing.

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