ELUCIDATION OF QUORUM QUENCHING AND MUTUALISTIC INTERACTION OF GREEN MICROALGA *Chlorella* sp. AND *Bacillus* sp. BpChIAY FOR BIOCONTROL OF VIBRIOSIS IN AQUACULTURE

By

NUR AIN BINTI YAHYA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

July 2020

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

ELUCIDATION OF QUORUM QUENCHING AND MUTUALISTIC INTERACTION OF GREEN MICROALGA *Chlorella* sp. AND *Bacillus* sp. BpChlAY FOR BIOCONTROL OF VIBRIOSIS IN AQUACULTURE

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July 2020

Chair : Associate Professor Natrah Fatin Mohd Ikhsan, PhD
Institute : Bioscience

Vibriosis are among the bacterial diseases that lead to massive mortality in cultured marine fish and shellfish limiting the sustainable development of aquaculture production. It is widely reported that *Vibrio* regulates its virulence factors through quorum sensing (QS), a cell-to-cell communication system mediated by small signal molecules produced by the pathogen in response to population density. Therefore, inhibiting QS, also known as quorum quenching (QQ) could prevent the regulation of virulence factors and is suggested as an alternative solution for biocontrol of vibriosis in aquaculture. The objectives of this study were to screen and identify QQ candidates from marine microalgae and their associated bacteria; to evaluate the effects of bacterial quenchers on microalgal growth and QQ activity in small flask (100 mL) and photobioreactor (100 L) scales; and to tentatively identify compounds with QQ properties. In the first part of this study, out of the 17 marine microalgae screened for QQ activity, only *Chlorella* sp. showed QQ activity by inhibiting the acyl-homoserine lactone (AHL) regulated phenotypes of purple violacein production in QS reporter *Chromobacterium violaceum* CV026 and autoinducer-2 (AI-2) regulated phenotypes of bioluminescence in *Vibrio campbellii* BB120 and its double mutant strain *V. campbellii* JMH597 without inhibiting the reporters' growth. Meanwhile, seven bacterial strains were successfully isolated from *Chlorella* sp., *Pavlova* sp., *Spirulina* sp. and *Nannochloropsis* sp. using *N*-hexanoyl-L-homoserine lactones (HHLs) enrichment method. Three of the bacteria, identified as *Bacillus* sp. BpChlAY, *Bacillus* sp. BpNofAY and *Bacillus* sp. BpSpiAY isolated from *Chlorella* sp., *N. oculata* and *Spirulina* sp., respectively, inhibited purple production of *C. violaceum* CV026, without affecting the reporter's growth. The cells of *Bacillus* sp. BpChlAY, *Bacillus* sp. BpNofAY and *Bacillus* sp. BpSpiAY were able to fully degrade 10 ppm of HHLs within 6 to 9 hours. However, none of their supernatants showed degradation activity. *In vivo* challenge test using *Artemia franciscana* as a model organism was carried out to evaluate the effects

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of *Chlorella* sp., *Bacillus* sp. BpChlAY, *Bacillus* sp. BpNofAY and *Bacillus* sp. BpSpiAY towards *Artemia* survival when challenged with QS-dependent pathogen *V. campbellii* BB120. The results showed that the live cells of *Chlorella* sp., live cells of *Bacillus* sp. BpChlAY and the combination of *Chlorella* sp. and *Bacillus* sp. BpChlAY significantly (P<0.05) improved *Artemia* survival towards *V. campbellii.* It is interesting to note that the *Bacillus* sp. BpChlAY was capable to reduce AHL and AI-2 regulated luminescence of the double mutant QS reporter *V. campbellii* JMH612 and *V. campbellii* JMH597, respectively. In the second part of this study, a co-culture experiment between *Chlorella* and each of the *Bacillus* sp. BpChlAY, *Bacillus* sp. BpNofAY and *Bacillus* sp. BpSpiAY was conducted in 100 mL culture volume for 14 days to determine the effects of bacterial quenchers towards *Chlorella* growth and its QQ activity. Out of the three bacterial strains, *Bacillus* sp. BpChlAY enhanced the *Chlorella* growth compared to *Bacillus* sp. BpNofAY and *Bacillus* sp. BpSpiAY. However, no QS degradation was observed in *C. violaceum* CV026 using the extracts from all treatments of the small-scale co-culture. Due to the positive effects towards *Artemia* survival and *Chlorella* growth, *Bacillus* sp. BpChlAY was co-cultured further with *Chlorella* in photobioreactor scale (100 L culture volume) for 45 days. The results showed that the addition of *Bacillus* sp. BpChlAY led to an increase in *Chlorella* growth up to 1.8-fold in terms of dry weight and 3.9-fold in both *Chlorella* cell density and *in vivo* chlorophyll fluorescence. The QQ activity of *Chlorella* was also improved in the presence of *Bacillus* sp. BpChlAY compared to *Chlorella* without *Bacillus* sp. BpChlAY after 45 days cultivation at the late stationary growth phase. No difference in QQ activity with or without the co-cultivation of the microalga and the bacteria at stationary stage of 25 days culture. In the last part of this study, the extracts of *Chlorella* from thin layer chromatography fraction (Chapter 3); and from photobioreactor (Chapter 4) with and without *Bacillus* sp. BpChlAY that were harvested at four different algal growth phases of lag, log, stationary and late stationary were tentatively identified using Ultra-High Performance Liquid Chromatography Electrospray Ionization Mass Spectrometry (UHPLC-ESI-MS). It was observed that there are no differences between the metabolite identities from the two *Chlorella* treatments. However, analysis of UHPLC-ESI-MS showed apparent molecular and fragment ions, which were consistent with 25 tentative metabolites identified and belonged to classes of tripeptides, lipid and lipid-like molecules, benzenoids, flavonoids, carbohydrates, lactones, quinolines and benzimidazoles. It is therefore concluded that both *Chlorella* and *Bacillus* sp. BpChlAY exhibited probiotic characteristics through AHL and AI-2 degradation activities, and are able to protect *A. franciscana* from *V. campbellii*. The bacteria also promote the *Chlorella* growth and enhanced QQ activity particularly at a later stage. The identification of *Chlorella* extracts with and without *Bacillus* sp. BpChlAY showed that it consisted of a wide range of biologically active compounds' classes. The combination of both partners, thus, constitute natural QQ probiotics that could be used to control vibriosis in aquaculture.

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PENCIRIAN PERENCAT KUORUM DAN INTERAKSI MUTUALISTIK MIKROALGA HIJAU *Chlorella* sp. DAN *Bacillus* sp. BpChlAY UNTUK KAWALAN BIOLOGI VIBRIOSIS DI AKUAKULTUR

Oleh

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Vibriosis merupakan salah satu penyakit terkait bakteria yang menyebabkan kematian besar-besaran pada ikan laut dan kerang-kerangan membataskan perkembangan lestari hasil akuakultur. Telah meluas dilaporkan bahawa *Vibrio* mengawalatur faktor virulennya melalui pengesanan kuorum (QS), satu sistem komunikasi sel ke sel melalui isyarat molekul kecil yang dihasilkan oleh patogen tersebut sebagai tindak balas terhadap kepadatan populasinya. Oleh itu, menghalang QS, juga dikenal sebagai perencat kuorum (QQ) dapat mencegah pengawalaturan faktor virulen dan dicadangkan sebagai penyelesaian alternatif untuk biokawalan vibriosis di akuakultur. Objektif kajian ini adalah untuk menyaring dan mengenalpasti calon QQ daripada mikroalga marin dan bakteria yang berasosiasi dengannya; untuk menilai kesan bakteria QQ terhadap pertumbuhan dan aktviti QQ mikroalga dalam skala kecil (100 mL) dan fotobioreaktor (100 L); dan untuk mengenalpasti secara tentatif sebatian dengan ciri QQ. Dalam bahagian pertama kajian ini, daripada 17 mikroalga marin yang disaring untuk aktiviti QQ, hanya *Chlorella* sp. menunjukkan aktiviti QQ dengan merencat penghasilan fenotip ungu violasin yang dikawalatur oleh lakton homoserin-asil (AHL) strain pelapor QS, *Chromobacterium violaceum* CV026 dan bioluminasi yang dikawalatur oleh autoinducer-2 (AI-2) *Vibrio campbellii* BB120 dan strain mutan bergandanya *V. campbellii* JMH597 tanpa merencat pertumbuhan strain pelapor QS tersebut. Sementara itu, tujuh strain bakteria berjaya dipencilkan daripada *Chlorella* sp., *Pavlova* sp., *Spirulina* sp. dan *Nannochloropsis* sp. menggunakan kaedah pengkayaan N-hexanoyl-L-homoserine lactone (HHL). Tiga daripada bakteria tersebut, dikenalpasti sebagai *Bacillus* sp. BpChlAY, *Bacillus* sp. BpNofAY dan *Bacillus* sp. BpSpiAY yang masing-masing dipencilkan daripada *Chlorella* sp.*, N. oculata* dan *Spirulina* sp.*,* merencat penghasilan ungu *C. violaceum* CV026 tanpa mempengaruhi pertumbuhan strain pelapor QS tersebut. Sel *Bacillus* sp. BpChlAY, *Bacillus* sp. BpNofAY dan *Bacillus* sp. BpSpiAY berupaya mendegradasi sepenuhnya 10 ppm HHL dalam masa 6 hingga 9 jam. Akan tetapi, supernatan bakteria tersebut

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tidak menunjukkan sebarang aktiviti degradasi. Ujian ketahanan secara *in vivo* menggunakan *Artemia franciscana* sebagai organisma model dilakukan untuk menilai kesan *Chlorella* sp.*, Bacillus* sp. BpChlAY, *Bacillus* sp. BpNofAY dan *Bacillus* sp. BpSpiAY terhadap ketahanan *Artemia* semasa dicabar dengan patogen bersandar-QS *V. campbellii* BB120. Hasil kajian menunjukkan bahawa sel hidup *Chlorella* sp., sel hidup *Bacillus* sp. BpChlAY dan gabungan *Chlorella* sp. dan *Bacillus* sp. BpChlAY meningkatkan ketahanan *Artemia* terhadap *V. campbellii* secara signifikan (P<0.05). Menarik untuk dikatakan bahawa *Bacillus* sp. BpChlAY berupaya mengurangkan luminasi-terkawalatur AHL dan AI-2, masing-masing oleh mutan berganda *V. campbellii* JMH612 dan *V. campbellii* JMH597. Dalam bahagian kedua kajian ini, eksperimen ko-kultur di antara *Chlorella* sp. dengan setiap satu *Bacillus* sp. BpChlAY, *Bacillus* sp. BpNofAY dan *Bacillus* sp. BpSpiAY dijalankan dalam 100 mL isipadu kultur selama 14 hari untuk menentukan kesan bakteria QQ terhadap pertumbuhan dan aktiviti QQ *Chlorella* sp. Daripada tiga strain bakteria tersebut, *Bacillus* sp. BpChlAY meningkatkan pertumbuhan *Chlorella* sp. berbanding dengan *Bacillus* sp. BpNofAY dan *Bacillus* sp. BpSpiAY. Akan tetapi, ekstrak daripada kesemua rawatan dalam ko-kultur skala kecil tidak menunjukkan degradasi QS terhadap *C. violaceum* CV026. Disebabkan kesan positif terhadap ketahanan hidup *Artemia* dan pertumbuhan *Chlorella* sp., *Bacillus* sp. BpChlAY selanjutnya telah di ko-kultur dengan *Chlorella* sp. dalam skala fotobioreaktor (100 L isipadu kultur) selama 45 hari. Hasil menunjukkan penambahan *Bacillus* sp. BpChlAY membawa kepada peningkatan pertumbuhan *Chlorella* sp. sebanyak 1.8 kali ganda berat kering dan 3.9 kali ganda dalam kedua-dua ketumpatan sel dan fluoresen klorofil *in vivo Chlorella* sp. Aktiviti QQ *Chlorella* sp. juga bertambah baik dengan kehadiran *Bacillus* sp. BpChlAY berbanding dengan kultur *Chlorella* sp. tanpa *Bacillus* sp. BpChlAY selepas pengkulturan selama 45 hari pada fasa pertumbuhan lewat pegun. Tiada perbezaan dalam aktiviti QQ dengan atau tanpa ko-kultur mikroalga dan bakteria pada kultur hari ke-25 fasa pegun. Dalam bahagian terakhir kajian ini, ekstrak *Chlorella* sp. daripada fraksi kromatografi lapisan nipis (Bab 3); dan daripada kultur fotobioreaktor (Bab 4) dengan atau tanpa *Bacillus* sp. BpChlAY yang dituai pada empat fasa pertumbuhan berbeza iaitu pada lag, log, pegun dan lewat pegun telah dikenalpasti secara tetntatif menggunakan *Ultra-High Performance Liquid Chromatography Electrospray Ionization Mass Spectrometry* (UHPLC-ESI-MS). Didapati bahawa tiada perbezaan di antara identiti metabolit daripada kedua rawatan *Chlorella* sp. Walau bagaimanapun, analisis UHPLC-ESI-MS menunjukkan ion molekul dan ion serpihan yang jelas dan konsisten dengan 25 metabolit tentatif yang telah dikenalpasti dan tergolong dalam kelas tripeptida, lipid dan molekul seperti lipid, benzenoid, flavonoid, karbohidrat, lakton, quinolin dan benzimidazol. Oleh itu, dapat disimpulkan bahawa kedua-dua *Chlorella* sp. dan *Bacillus* sp. BpChlAY menunjukkan ciri probiotik melalui aktiviti degradasi AHL dan AI-2, dan mampu melindungi *Artemia* daripada *Vibrio campbellii.* Bakteria juga menggalakkan pertumbuhan *Chlorella* sp. dan meningkatkan aktiviti QQ terutamanya pada lewat pertumbuhan. Pengenalpastian ektrak *Chlorella* sp. dengan atau tanpa *Bacillus* sp. BpChlAY menunjukkan ia terdiri daripada pelbagai sebatian aktif biologi daripada kelas yang berbeza. Dengan ini, kombinasi kedua-dua *Chlorella* sp. dan *Bacillus* sp. BpChlAY membentuk probiotik QQ semulajadi yang boleh digunakan untuk mengawal vibriosis di akuakultur.

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"In memory of my beloved *arwah mak* and *ayah.* Indeed, we belong to Allah, and indeed to Him we will return. Al-fatihah"

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LIST OF ABBREVIATIONS

AF	Algal filtrate
ACN	Acetonitrile
AHLs	Acyl homoserine lactones
AHPND	Acute Hepatopancreatic Necrosis Disease
AIs	Autoinducers
AI-1	Autoinducer 1
AI-2	Autoinducer 2
AMR	Anti-microbial resistance
ANOVA	Analysis of variance
BFT	Biofloc technology
BLAST	Basic Local Alignment Search Tool
bp	Basepair
°C	Degree Celsius
^{12}C	Carbon-12
^{13}C	Carbon-13
^{14}C	Carbon-14
CAI-1	Cholera Autoinducer 1
cfu	Colony forming unit
^{35}Cl	Clorine-35
^{37}Cl	Clorine-37
CO_2	Carbon dioxide

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DAD	Diode array detector
DCM	Dichloromethane
DHA	Docosahexaenoic acid
DKPs	Diketopiperazines
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOF	Department of Fisheries
DOM	Dissolved organic matters
DSFs	Diffusible signal factors
EA	Ethyl acetate
EF	Extracellular fraction
EMS	Early mortality syndrome
EPA	Eicosapentaenoic acid
EPP	Entry Point Projects
ESI	Electrospray ionization
EtOAc	Ethyl acetate
FAO	Food and Agriculture Organization
g	Gram
h	Hour
HAI-1	Harveyi autoinducer 1
HESI	Heat electrospray ionization
HHL	*N*-hexanoyl-L-homoserine lactones
HPLC	High performance liquid chromatography

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HSL	Homoserine lactone
IF	Intracellular fraction
IQS	Integrated QS system
Kb	Kilobase
kg	Kilogram
L	Litre
LB	Luria-Bertani
LC	Liquid chromatography
LCMS	Liquid chromatography-mass spectrometry
LS	Late stationary
MA	Marine agar
MAS	Motile *Aeromonas* Septicaemia
MB	Marine broth
MeOH	Methanol
mg	Milligram
min	Minute
mL	Millilitre
mm	Millimetre
mM	Milimolar
MOA	Ministry of Agriculture and Agro-food Industry
MOPS	3-Morpholinopropane-1-sulfonic acid
MS	Mass spectrometry
m/z	Mass-to-charge ratio

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Na	Not applicable
NAP	National Agro-food Policy
Nd	Not determined
NKEA	National Key Economic Area
NOAA	National Oceanic and Atmospheric Administration
OECD	Organization for Economic Cooperation and Development
ONPG	ortho-Nitrophenyl-β-galactoside
OOHL	*N*-3-oxo-octanoyl homoserine lactones
PBR	Photobioreactor
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
ppm	Part per million
PQS	*Pseudomonas* quinolone signal
PSM	Phenol soluble modulins
PUFAs	Polyunsaturated fatty acids
QQ	Quorum quenching
QS	Quorum sensing
QSIs	Quorum sensing inhibitors
R_f	Retention factor (Ds/Df)
rpm	Rotation per minute
RLU	Relative luminescence unit

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RT	Retention times
SAS	Statistical Analysis Software
sd	Standard deviation
se	Standard error
t_R	Retention time
TIC	Total ion chromatogram
TLC	Thin layer chromatography
TSA	Tryptic soy agar
µm	Micrometer
UHPLC-MS	Ultra-high performance liquid chromatography mass spectrometry
UHPLC-ESI MS	Ultra-High Performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry
UHPLC-MSMS	Ultra-high performance liquid chromatography tandem mass spectrometry
UHPLC-DAD-ESI-MS Orbitrap	Ultra-high Performance Liquid Chromatography with Diode Array Detection and Electrospray Ionization source Mass Spectrometry with Orbitrap analyser
UHPLC-ESI-Orbitrap MS	Ultra-high Performance Liquid Chromatography-Electrospray Ionization-Orbitrap Mass Spectrometry
USD	United States Dollar
UV	Ultraviolet
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The demand for fishery products is driven by population and income growth (Subasinghe 2015). Along with the increasing of global population, Malaysia's population is likely to rise as well based on the yearly increasing trend from 32.0 million to 32.4 million and to 32.6 million in 2017, 2018 and 2019, respectively (United Nations 2019; Department of Statistics Malaysia 2019). To cater for future demand, sustainable intensification of food production will undeniably be a major challenge. The high demand for protein food source has caused aquaculture sector together with capture fisheries to expand rapidly since the past few years. In fact, FAO statistics showed that aquaculture is one of the fastest-growing food producing industry with an average increase rate of 7.8% worldwide, between 1990 and 2010, higher as compared to other industries of poultry (4.6%), pork (2.2%), dairy (1.4%), beef (1.0%) and grains (1.4%), within the same time frame (Troell et al. 2014). In addition, due to the fact that coastal fishery resources have been overexploited as well as climate change and global warming that impacted marine life, global capture fishery production was relatively static since the late 1980s, and thus, aquaculture is projected to reach 109 million tonnes in 2030, for the continuing growth in the fish supply for human consumption, which is crucial in meeting FAO's goal of a world without hunger and malnutrition (FAO 2018; 2020).

In Malaysia, efforts to empower aquaculture industry are aimed at increasing aquaculture output by 50% in 2020 to balance between aquaculture and capture fisheries. Therefore, aquaculture is listed amongst the 16 Agro-food's Entry Point Projects (EPP) of the National Key Economic Area (NKEA). In 2010, Malaysian Government through the Ministry of Agriculture and Agro-food Industry (MOA) has introduced the National Agro-food Policy (NAP) from 2011-2020 to ensure the national food security and boost revenues from the agricultural sector, including aquaculture (Othman et al. 2017; Dardak 2019). To this, Department of Fisheries (DOF) has initiated the Aquaculture Industrial Zone (AIZ) Programme for coastal zone management where suitable areas are identified and allocated for aquaculture use with aim of increasing fishes' output by culturing of various types of high value aquatic species at commercial scales (Yusoff 2015; Othman et al. 2017). Thus, as the fastest growing food-producing sector and with the government support, aquaculture is one of the key players in ensuring sustainable development of food reservoir and may shape future food security particularly in Malaysia and generally worldwide.

However, the fluctuation of Malaysian aquaculture production which recorded a huge declining within 2012 till 2016, as well as decrease in 2018 has become a major constraint in order to cater for future food demand (Figure 1.1). One of the

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main factor is the occurrence of disease outbreaks that led to a significant economic loss due to high mortality and low yield quality of aquatic animals. Among microbes that caused diseases, bacteria are considered the common pathogens that frequently infected cultured aquatic animals, followed by viruses, parasites and fungi (Wei & Wee 2014). To name a few, bacterial diseases such as vibriosis, streptococcosis, Motile *Aeromonas* Septicaemia (MAS) and Acute Hepatopancreatic Necrosis Disease (AHPND) are among the common diseases in Malaysia that infect cultured fishes and crustaceans (Shariff 1998; Wei & Wee 2014; Zamri-Saad et al. 2014; Chiew et al. 2019). In fact, Chiew et al. (2019) reported that within 20 years of disease reporting in Malaysia, several bacterial and viral diseases were still found to persist in farms. In this study, focus has been made on the disease caused by *Vibrio* sp. particularly vibriosis that leads to massive mortality of cultured shrimp, fish and shellfish. Various factors lead to vibriosis occurrence especially the source of fish, the environmental factors including farm management and water quality as well as virulence factors of the pathogenic *Vibrio* (Ina-Salwany et al. 2019).

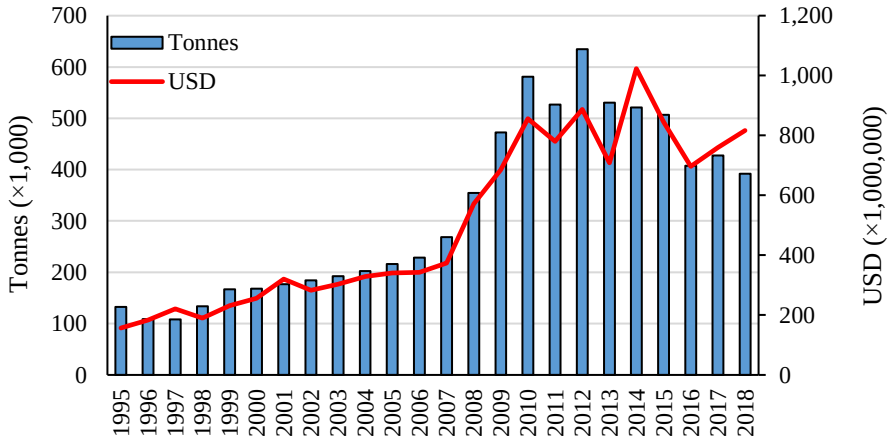

Figure 1.1: Total aquaculture production for Malaysia (tonnes and USD)

[Source: Organization for Economic Cooperation and Development (OECD 2020)]

1.2 Problem statement

Intensive farming has become a common practice to meet the seafood-based protein demand in aquaculture. Intensification promoted pathogens growth that leads to disease occurrence and could be a major threat to the aquaculture sustainability (Barman et al. 2013). The use of synthetic chemicals and antibiotics to overcome diseases has achieved partial success but lack of awareness and control of using them has raised other serious issues involving public health (Defoirdt et al. 2011b; Barman et al. 2013; Cabello et al. 2016). For instance, the capacity of antibiotics to inhibit the growth of bacteria could induce selection pressure and promoting anti-microbial resistance (AMR) among bacterial communities in order for them to survive in the unfavourable condition

(Romero et al. 2012). Even worse, the massive use of antibiotics accelerates and spread the emergence of AMR pathogens, since the genes responsible for AMR can travel vertically or horizontally across ecosystems, from the environment to human (Bhushan et al. 2016; Miranda et al. 2018). Tendencia and de la Pena (2001; 2002) isolated bacteria (mostly *Vibrio* sp.) in different shrimp ponds, and found that *Vibrio* isolated from ponds with previous history usage of antibiotic showed a broad-spectrum antibiotic resistance compared to *Vibrio* isolated from ponds without any antibiotic treatment.

On top of that, vaccination has been developed with some success depending on the specific pathogens such as *Vibrio* strains, however, the antigenic diversity of *Vibrio* strains and their serotypes resulting in slow progress of vaccine development due to vaccine limitation to elicit protection against multiple *Vibrio* infection (Li et al. 2010; Li et al. 2014; Ina-Salwany et al. 2019). Biofloc technology (BFT), a crop intensification with the use of microbiota in the fish culture system to maintain water quality resulting in minimum or zero water exchange seems promising but requires start-up period, alkaline supplement to maintain water pH, pollution potential from the increasing of nitrate accumulation and is inconsistent performance based on availability of sunlight (Dauda 2019; Jung et al. 2020). Therefore, an alternative practice is urgently needed to control infections caused by bacterial pathogens.

1.3 Significance of study

Many researchers reported that aquaculture bacterial pathogens use quorum sensing (QS), a communication between bacterial cells via small signal molecules, to control virulence factor expression, some of which are extracellular toxin, metalloprotease and haemolysin (Ruwandeepika et al. 2012; Zhao et al. 2014; Defoirdt 2018). These virulence-associated factors regulated by pathogens' QS are required in order for them to colonise and infect their host, which will harm and cause disease to the host (Rutherford & Bassler 2012). Thus, preventing pathogens from producing the harmful factors becomes an interesting way for disease control. This is known as QS inhibition or quorum quenching (QQ). Where contrast to antibiotics, the QQ only targets bacterial communication, thereby making them in 'mute' or 'disarmed' state, preventing them from infecting their host. This in time will allow the host natural immune system to clear the disarmed pathogen from the disease site (Dickey et al. 2017; Defoirdt 2018). Importantly, QQ therapy can bypass the side effects of antibiotics that inhibit the growth of many beneficial bacteria. These beneficial bacteria play an important role including inducing food digestion in animal gut, promoting immune system development and resisting colonization of pathogenic bacteria (Dickey et al. 2017).

The first QQ compound was reported from seaweed (macroalgae), known as halogenated furanone (Manefield et al. 1999). Unfortunately, furanone was found unsuitable for human use due to its instability and high toxic (Cotar 2013), leading to the search of more QQ compounds from other sources. Strategies to

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control pathogen load need to be further developed especially in marine larviculture to improve the overall production of adult organisms. In general, this study was focused on finding potential QQ properties from marine sources, especially from microalgae and their associated bacteria. As the basis of marine food chain, microalgae play a crucial nutritional role for the life cycle of marine organisms that are widely reported to improve fish, shrimp, and shellfish larval growth, survival and feed ingestion, as well as for live-prey cultivation and enrichment of live food (Sirakov et al. 2015; Ahmad et al. 2018; Han et al. 2019). Microalgae have been shown to interfere QS signal molecule in different biosensor strains, including the aquaculture pathogen, *Vibrio harveyi* (Natrah et al. 2011). Flandez (2011) also demonstrated that the co-culture of QQ bacteria to specific microalgae improved survival of *Artemia* and mussel. The addition of *Bacillus* sp. isolated from an open culture of microalga *Chaetoceros muelleri,* to the rearing water of giant river prawn larvae, *Macrobrachium rosenbergii,* also improved its survival against QS-dependent pathogen, *Vibrio campbellii,* without affecting the larval growth (Pande et al. 2015). This suggests that by adding QS inhibitor (with probiotic potential) could possibly protect the aquatic organisms from pathogenic bacteria. Moreover, microalgae and bacteria often interact with each other mainly for nutrient exchange, signal transduction and gene transfer, which indirectly shape aquatic communities and influence biogeochemical cycles in the natural environment (Kouzuma & Watanabe, 2015). The win-win situation of algae and bacteria has attracted an interest to further elucidate their relationships in terms of growth performance and metabolites involved during co-culture. Therefore, the QQ strategy using microalgae and/ or bacteria as probiotics are proposed as an alternative solution for bacterial disease biocontrol. The early stage of live food feeding using QQ microalga and QQ bacteria was focused, with respect to possible applications to control vibriosis in marine larviculture.

1.4 Objectives

1. To screen and identify potential quorum quenchers from microalgae and microalgae-associated bacteria
2. To determine the effects of selected bacterial quenchers on microalgal growth and quorum quenching activity
3. To identify metabolites produced by microalga with and without bacterial quencher using spectrometric analysis

1.5 Hypotheses

1. Microalgae and microalgae-associated bacteria could be source of quorum quenchers discovery
2. Bacterial quenchers improve microalgal growth and quorum quenching activity
3. There are differences on metabolites classes produced by microalga with and without bacterial quencher

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