



UNIVERSITI PUTRA MALAYSIA

***ANTI-PATHOGENIC ACTIVITIES OF *Gracilaria changii* (Xia et Abbott)
AND EFFECTS OF ITS ASSOCIATED BACTERIA ON GROWTH
PROMOTING PROPERTIES***

NURUL IZZATI MUTHAR

IB 2018 18



**ANTI-PATHOGENIC ACTIVITIES OF *Gracilaria changii* (Xia et Abbott)
AND EFFECTS OF ITS ASSOCIATED BACTERIA ON GROWTH
PROMOTING PROPERTIES**

By

NURUL IZZATI MUTHAR

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Master of
Science**

February 2018

COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ANTI-PATHOGENIC ACTIVITIES OF *Gracilaria changii* (Xia et Abbott) AND THE EFFECTS OF ITS ASSOCIATED BACTERIA ON GROWTH PROMOTING PROPERTIES

By

NURUL IZZATI MUTHAR

February 2018

Chairman: Natrah Fatin Mohd Ikhsan, PhD

Faculty: Institute of Bioscience

Seaweed contains various bioactive compounds that contributed to the pharmaceutical and food industries which demand high seaweed production. Limitation of healthy seedstock might lead to the reduction of seaweed production and the study of seaweed-bacteria interaction could potential enhance the growth of the organism. The interest in bacterial populations living in association with seaweed has increased during recent decades. Bacteria associated with seaweed secrete various beneficial compounds which can act as defense mechanism and also regulate the morphogenesis of seaweed. Thus, a study on the beneficial interaction between seaweed and bacteria especially on the growth promoting properties is crucial. The aim of this study was to determine the anti-pathogenic activities of *Gracilaria changii* and the effects of its associated bacteria on the growth promoting properties. The first study was to investigate anti-quorum sensing (anti-QS) and anti-biofilm activities from *G. changii* extracts. The anti-QS activities were screened using disc diffusion assay with mutant *Chromobacterium violacein* (CV026) strain as a quorum sensing (QS) biosensor strain. It was observed that only *G. changii* crude extracts at 240,000 ppm significantly ($P < 0.05$) inhibited the QS activity. Using thin layer chromatography (TLC), three out of six *G. changii* compounds separation showed significant ($P < 0.05$) QS inhibition zones after subjected to overlay assay. The results also showed that *G. changii* extracts inhibited ($P < 0.05$) the biofilm of pathogenic strain *Vibrio campbellii* BB120 at the concentration of 1 ppm after 24 hours incubated with *G. changii* extracts. In the second study, associated bacteria isolated from *G. changii* with anti-QS and anti-bacterial activities were characterized. A total of 28 different bacteria were isolated from *G. changii* in which 23 strains were epiphytic bacteria and the remaining 5 strains were endophytic bacteria. There was no anti-QS activity observed from bacteria associated *G. changii* using both well plate and disc diffusion assays with CV026 as the biosensor. No anti-bacterial activity was also detected from all bacterial isolates towards the four tested pathogens; *Vibrio campbellii* (BB120), *V. parahaemolyticus* (ATCC17803), *V. anguillarum* (ATCC43313) and *V. alginolyticus*

(ATCC17749). The third study was conducted to determine the interaction between *G. changii* and the associated bacteria for growth promoting activities. Out of all the 28 bacteria isolated from the seaweed, BP-SW/7 which was identified as *Halomonas* sp. significantly ($P < 0.05$) promoted the highest *G. changii* bud formation. The bacterial density of *Halomonas* sp. also significantly ($P < 0.05$) increased from 10^7 to 10^9 CFU/mL after 15 days of co-culture with *G. changii* fragments. Moreover, the highest ($P < 0.05$) plant growth promoting hormone indole-3-acetic acid (IAA) production was also observed from the bacterial isolates BP-SW/7, *Halomonas* sp. after three days incubations. The results of this study showed that there is a symbiotic relationship between seaweed and certain associated bacteria particularly for growth promotions of both the seaweed and associated bacteria. Furthermore, *G. changii* could be an interesting source for biologically active compounds that may be applied for disease therapy instead commercial antibiotics.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

AKTIVITI ANTI-PATOGENIK OLEH *Gracilaria changii* (Xia et Abbott) DAN KESAN BAKTERIA YANG BERKAITAN ATAS SIFAT YANG MENGGALAKKAN PERTUMBUHAN

Oleh

NURUL IZZATI MUTHAR

Februari 2018

Pengerusi: Natrah Fatin Mohd Ikhsan, PhD
Faculti: Institut Biosains

Rumpai laut mengandungi pelbagai sebatian bioaktif menyumbang kepada industri farmaseutikal dan makanan yang menuntut pengeluaran rumpai laut yang tinggi. Benih yang sihat yang terhad boleh mengakibatkan pengurangan pengeluaran rumpai laut dan kajian interaksi rumpai laut-bacteria berpotensi meningkatkan pertumbuhan organisma. Kepentingan populasi bacteria yang hidup bersekutu dengan rumpai laut telah meningkat dalam beberapa dekad kebelakangan ini. Bacteria yang dikaitkan dengan rumpai laut menghasilkan pelbagai sebatian bermanfaat yang boleh bertindak sebagai mekanisme pertahanan dan juga mengawal morphogenesis rumput laut. Oleh itu, satu kajian tentang interaksi yang bermanfaat antara rumput laut dan bacteria terutama pada sifat yang menggalakkan pertumbuhan adalah penting. Tujuan kajian ini adalah untuk menentukan aktiviti anti-patogenik oleh *Gracilaria changii* dan kesan bacteria yang berkaitan atas sifat yang menggalakkan pertumbuhan. Kajian pertama adalah untuk mengkaji aktiviti anti-kuorum penginderaan dan anti-biofilm daripada ekstrak *G. changii*. Aktiviti anti-kuorum penginderaan telah disaring menggunakan ujian penyebaran cakera dengan strain *Chromobacterium violaceum* (CV026) mutan sebagai biosensor kuorum penginderaan. Telah dilihat bahawa hanya ekstrak mentah *G. changii* pada kepekatan tinggi ($P < 0.05$) menghalang aktiviti kuorum penginderaan. Menggunakan kromatografi lapisan nipis (TLC), tiga daripada enam sebatian *G. changii* menunjukkan zon terencat kuorum penginderaan yang signifikan ($P < 0.05$) selepas mengenakan ujian overlay. Hasilnya juga menunjukkan bahawa ekstrak *G. changii* menghalang ($P < 0.05$) pertumbuhan biofilm patogen *Vibrio campbellii* BB120 pada kepekatan 1ppm selepas 24 jam dikultur bersama ekstrak *G. changii*. Dalam kajian kedua, bacteria yang diasingkan dari *G. changii* dengan aktiviti anti kuorum penginderaan dan anti-bacteria dicirikan. Sejumlah 28 bacteria yang berbeza telah diasingkan dari *G. changii* di mana 23 jenis bacteria epifit dan 5 bacteria yang lain adalah bacteria endofit. Tiada aktiviti anti penginderaan kuorum yang diperhatikan dari bacteria yang berkaitan *G. changii* menggunakan kedua-dua plat pancang dan percikan resapan cakera dengan CV026 sebagai

biosensor. Tiada aktiviti antibakteria juga dikesan dari semua bakteria yang diasingkan ke atas empat patogen yang diuji; *Vibrio campbellii* (BB120), *Vibrio parahaemolyticus* (ATCC17803), *Vibrio anguillarum* (ATCC43313) dan *Vibrio alginolyticus* (ATCC17749). Kajian ketiga dijalankan untuk menentukan interaksi antara *G. changii* dan bakteria yang berkaitan dengan aktiviti yang menggalakkan pertumbuhan. Daripada semua 28 bakteria yang diasingkan dari rumpai laut, BP-SW / 7 yang dikenal pasti sebagai *Halomonas* sp. dengan ketara ($P < 0.05$) menggalakkan pembentukan putik *G. changii* tertinggi. Kepekatan bakteria *Halomonas* sp. juga ketara ($P < 0.05$) meningkat dari 10^7 hingga 10^9 selepas 15 hari dikultur bersama dengan keratan *G. changii*. Selain itu, hormon yang menggalakkan pertumbuhan tumbuhan asid indole-3-asetik (IAA) yang tertinggi ($P < 0.05$) juga diperhatikan dari bakteria yang diasingkan BP-SW / 7, *Halomonas* sp. selepas tiga hari inkubasi. Keputusan kajian ini menunjukkan terdapat hubungan simbiotik antara rumpai laut dan bakteria yang berkaitan terutamanya untuk menggalakkan pertumbuhan kedua-dua rumpai laut dan bakteria yang berkaitan. Tambahan pula, *G. changii* boleh menjadi sumber yang menarik untuk sebatian aktif biologi yang boleh digunakan untuk terapi penyakit selain antibiotik komersial.

ACKNOWLEDGEMENT

I would like to express my utmost gratitude and appreciations to my supervisor, Dr Natrah Fatin Mohd Ikhsan for her supervision, assistance and inspirations to accomplish this project. Special thanks to my co-supervisor, Assoc. Prof. Dr. Mutah Harah Zakaraia @ Ya and Assoc. Prof. Dr. Yaya Rukayadi for their guidance, advice and support throughout my study.

I would also like extend my gratitude and thanks to all the member of Bioproduct Aquatic Laboratory, Faculty of Aquaculture, kak Ain, kak Sherry, kak Chen, kak Arina, kak Izzah, Sarmilla, Daus, Aini, Tan and Atifa. Special thanks to my fyp student, Syamimi for her never ending help and support.

My deepest thanks to my family especially my parent, Muthar B. Ibrahim and Che Yam Bt Rahman for their motivation and support in completing my master.

My inmost appreciation my best friends Nik Suzana, Syaheerah, Atifa and Jennifer Ebit for their advice, support, motivation, late night company and wise words.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Natrah Fatin Mohd Ikhsan, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Muta Harah Binti Zakaria @ Ya, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Yaya Rukayadi, PhD

Associate Professor
Faculty of Food Science
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Nurul Izzati Muthar (GS 43957)

Declaration by Members of Supervisory Committee

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature :

Name of Chairman of
Supervisory Committee :

Natrah Fatin Mohd Ikhsan

Signature :

Name of Member of
Supervisory Committee :

Muta Harah Binti Zakaria @ Ya

Signature :

Name of Member of
Supervisory Committee :

Yaya Rukayadi

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xiii
LIST OF FIGURES		xiv
LIST OF ABBREVIATIONS		xv
CHAPTER		
1	INTRODUCTION	1
	1.1 Background	1
	1.2 Problem Statement	2
2	LITERATURE REVIEW	3
	2.1 Seaweed	3
	2.2 Potential Applications of Seaweed	4
	2.3 <i>Gracilaria changii</i>	5
	2.3.1 Nomenclature and Taxonomy	5
	2.3.2 Life Cycle of <i>Gracilaria</i> sp.	5
	2.3.3 Identification of <i>Gracilaria changii</i>	7
	2.4 Anti-Quorum Sensing Activity from Seaweed Extracts	9
	2.5 Seaweed-Bacteria Interaction	10
	2.5.1 Anti-pathogenic Activities from Bacteria Associated with Seaweed	11
	2.5.2 Growth Promoting Activity from Seaweed-Bacteria	15
3	DETERMINATION OF ANTI-QUORUM SENSING AND ANTI-BIOFILM ACTIVITIES FROM RED SEAWEED, <i>Gracilaria changii</i>	16
	3.1 Introduction	16
	3.2 Materials and Methods	17
	3.2.1 Glasswares and Instruments Preparation	17
	3.2.2 Media Preparation	17
	3.2.3 <i>Gracilaria changii</i> Extracts Preparation	17
	3.2.3.1 Samples Collection	17
	3.2.3.2 Collection of <i>Gracilaria changii</i> Crude Extracts	17
	3.2.4 Anti-Quorum Sensing Activities from <i>Gracilaria changii</i> Extracts	18
	3.2.4.1 <i>Chromobacterium violacein</i>	18

	(CV026) preparation	
	3.2.4.2 Screening of Anti-Quorum Sensing Activity from <i>Gracilaria changii</i> Extracts	18
	3.2.5 Anti-Quorum Sensing Compound	19
	3.2.5.1 Partial Purification of the <i>Gracilaria changii</i> Crude Extracts using Thin-Layer Chromatography (TLC)	19
	3.2.5.2 Thin-Layer Chromatography (TLC) Overlay Assay	19
	3.2.6 <i>Vibrio campbellii</i> (BB120) Biofilm Inhibition by <i>Gracilaria changii</i> Extracts	19
	3.2.7 Statistical Analysis	20
3.3	Results	21
	3.3.1 Anti-Quorum Sensing Activities from <i>Gracilaria changii</i> Extracts	21
	3.3.2 Anti-Quorum Sensing Metabolites from <i>Gracilaria changii</i> Crude Extracts	22
	3.3.3 <i>Vibrio campbellii</i> Biofilm Inhibition from <i>Gracilaria changii</i> Extract	23
3.4	Discussion	24
3.5	Conclusion	26
4	EVALUATION OF <i>Gracilaria changii</i> ASSOCIATED BACTERIA WITH ANTI-PATHOGENIC ACTIVITIES	27
	4.1 Introduction	27
	4.2 Materials and Methods	28
	4.2.1 Media Preparation	28
	4.2.2 Anti-Quorum Sensing Activities from Bacteria Associated with <i>Gracilaria changii</i>	28
	4.2.2.1 Isolation of <i>Gracilaria changii</i> Associated Bacteria	28
	4.2.2.2 <i>Chromobacterium violacein</i> (CV026) Preparation	28
	4.2.2.3 N-Acyl Homoserine Lactones (AHL) Degradation Strain	28
	4.2.3 Anti-Bacterial Activities from Bacteria Associated with <i>Gracilaria changii</i>	29
	4.2.3.1 Disc Diffusion Assay	29
	4.2.3.2 Spot on Lawn	30
	4.2.3.3 Cross Streak Assay	30
4.3	Results	30
	4.3.1 Anti-Quorum Sensing Activities from Bacteria Associated with <i>Gracilaria changii</i>	30
	4.3.2 Anti-Bacterial Activities from Bacteria Associated with <i>Gracilaria changii</i>	33
4.4	Discussion	34

4.5	Conclusion	35
5	INTERACTION BETWEEN <i>Gracilaria changii</i> AND THE ASSOCIATED BACTERIA FOR GROWTH PROMOTING ACTIVITY	36
5.1	Introduction	36
5.2	Materials and Methods	37
5.2.1	Effects of Bacterial Cells on <i>Gracilaria changii</i> Protuberance Formation	37
5.2.2	Quantification of Indole-3-Acetic Acid (IAA) Production by Selected Bacteria	37
5.2.3	Plate Count Agar	37
5.2.4	Bacterial Identification	38
5.2.4.1	Gram Staining	38
5.2.4.2	Molecular Characteristic and Identification	38
5.2.4.2.1	DNA Extraction and Identification of Selected Bacterial Strains	38
5.2.4.2.2	Polymerase Chain Reaction (PCR)	39
5.2.4.2.3	Sequence Analysis	40
5.2.5	Statistical Analysis	40
5.3	Results	40
5.3.1	Effects of Bacterial Cells on <i>Gracilaria changii</i> Protuberance Formation	40
5.3.2	Quantification of Indole-3-Acetic Acid (IAA) Production by Selected Bacteria	43
5.3.3	Bacterial Plate Count	44
5.3.4	Identification of Indole-3-Acetic Acid (IAA) Producing Strain	44
5.4	Discussion	46
5.5	Conclusion	48
6	SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH	49
	REFERENCES	51
	APPENDICES	66
	BIODATA OF STUDENT	68
	PUBLICATION	69

LIST OF TABLES

Table		Page
2.1	Aquaculture Pathogenic Bacterial Strains	9
2.2	Bacteria Associated with Seaweed	12
3.1	Biofilm Assay with and without <i>Gracilaria changii</i> Extracts at Different Concentration	20
3.2	Inhibition zone (mm) of Anti-Quorum Sensing Activity from <i>Gracilaria changii</i> Extracts using Disc Diffusion Assay	22
3.3	R _f Values of <i>Gracilaria changii</i> Compound	22
3.4	Effects of <i>Gracilaria changii</i> Extracts on <i>Vibrio campbellii</i> (BB120) Biofilm after 24 h Incubation	23
4.1	Epiphytic Bacterial Strains Isolated from <i>Gracilaria changii</i>	31
4.2	Endophytic Bacterial Strains Isolated from <i>Gracilaria changii</i>	32
5.1	Effects of Epiphytic Bacterial Strain on Number of Protuberance Formation of <i>Gracilaria changii</i> after 4 Weeks Culture	41
5.2	Effects of Endophytic Bacterial Strain on Number of Protuberance Formation of <i>Gracilaria changii</i> after 4 Weeks Culture	41
5.3	Concentration of Indole-3-Acetic Acid (IAA) Produced by Selected Bacterial Strains	44
5.4	Colony Forming Unit (CFU) of the Inducing-Protuberance Formation Strain BP-SW/7 after 15 days Co-Culture with <i>Gracilaria changii</i> Fragment	45
5.5	Similarity Values for the Closest Relatives of 16S rRNA Gene Sequences Retrieved from Isolates	46

LIST OF FIGURES

Figure		Page
2.1	Life Cycle of <i>Gracilaria</i> sp.	6
2.2	Cystocarp Formation on Female Gametophyte	7
2.3	<i>Gracilaria changii</i> Physical Morphology	7
2.4	Common <i>Gracilaria</i> sp.	8
2.5	Overview of Beneficial (green) and Detrimental (red) Interactions between Seaweed and Bacteria	11
3.1	Clear Zone Indicates Anti-Quorum Sensing Activity Inhibition Zone from <i>Gracilaria changii</i> Extracts using Disc Diffusion Assay	21
3.2	R _f Values from Thin Layer Chromatography (TLC) Overlay Assay for QS Inhibition from <i>Gracilaria changii</i> Crude Extracts	23
4.1	Different Colour of Bacterial Cultures Isolated from <i>Gracilaria changii</i>	30
4.2	Clear Zone Indicates Anti-Quorum Sensing Activity Inhibition Zone from <i>Gracilaria changii</i> Associated Bacteria using Disc Diffusion Assay	32
4.3	Clear Zone Indicates Anti-Quorum Sensing Activity Inhibition Zone from <i>Gracilaria changii</i> Associated Bacteria using Well Plate Assay	33
4.4	Anti-Bacterial Activity from <i>Gracilaria changii</i> Associated Bacteria against Different Treatments	33
5.1	Schematic PCR Amplification using Two Sets of Primers	39
5.2	Protuberance Formation of <i>Gracilaria changii</i> at Different Treatments	42
5.3	Indole-3-Acetic Acid (IAA) Standard Curve	43
5.4	Colour Changes of Different Indole-3-Acetic Acid (IAA) Concentration	43
5.5	Gram Staining of Indole-3-Acetic Acid (IAA) Producing Bacterial Strains, <i>Halomonas</i> sp. BP-SW/7 under 1000X Magnification	45
5.6	The Agarose Gel Electrophoresis of PCR Amplified DNA Product of 16S rRNA Gene	45

LIST OF ABBREVIATIONS

AHL	N-Acyl homoserine lactones
BLAST	Basic Local Alignment Search Tool
CFU	Colony Forming Unit
DoF	Department of Fishery
EPS	Extracellular Polymeric Sunstances
FAO	Food and Agriculture Organization
FeCl ₃	Iron (III) chloride
HHL	N-hexanoyl-L-homoserine lactone
IAA	Indole-3-Acetic Acid
LBA	Luria-Bertani Agar
LBB	Luria-Bertani broth
HClO ₄	Perchloric acid
ppm	parts per million
psu	Practical salinity unit
QS	Quorum Sensing
QSI	Quorum Sensing Inhibition
RAPD	Rapid Amplified Polymorphic DNA
sp.	Species
TBE	Tris/Borate/EDTA
TLC	Thin Layer Chromatography
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth

CHAPTER 1

INTRODUCTION

1.1 Background

Seaweed cultivation is growing worldwide and expanding at 8% per year in the past decade (FAO, 2014). Scientist and entrepreneurs have created great interest in the use of high-quality agar and agarose extracted from *Gracilaria changii* (Phang et al., 1996). These agars are known as substitution for gelatin in food, cosmetic, and pharmaceutical applications. In the last two decades, seaweed production has increased and became a potential economic resource in the Asia-Pacific region, including Malaysia (Phang, 2006).

Nowadays, 96% of the world seaweed supply comes from aquaculture with approximately over 23 million tons of seaweed (dry weight) were produced in 2012 from aquaculture, worth over US\$6 billion (FAO, 2012; 2014). Seaweed industry in Malaysia contributed to the revenue of RM66 million in 2012 which is mainly from Sabah (DoF, 2012). This reflects that seaweed can be a profitable business for the country. Encouraged by this output, the government introduced seaweed mini-estate program to increase the seaweed productions through a more organized and integrated system in ensuring high quantity and quality seaweed production (PEMANDU, 2010).

In Malaysia, *Gracilaria* species is among the attractive species candidate for intensive culture (Norizan, 1999). However, maintenance of healthy seedstock continuous supplies is a critical issue in seaweed cultivation nowadays. To date, the seaweed seedstock has been supplied from the wild (Buschmann et al., 2008) and due to the physical variations, the seaweed is exposed to the various diseases. This make it more challenging to control the seed quality which may leads to a serious drop in the seaweed production (Rafael et al., 2015).

Interestingly, seaweed associated bacteria had been known to play an important role in the growth, development, morphogenesis and reproduction of the seaweed (Singh et al., 2011a). Seaweed-associated bacteria has been found could enhance the development and morphogenesis of seaweed compared to the axenic seaweed culture (Singh et al., 2011a) by producing bioactive compound and plant growth regulators (PGRs). This suggested that seaweed that cultured with associated bacteria will grow healthily and mature faster than seaweed which is left untreated. Hence, application of associated bacteria seems promising alternative to control and increase the production of seaweed.

Besides that, disease-related problem is also a major constraint to the aquaculture organisms thus threatens the development of the aquaculture industry (Bondad-Reantaso et al., 2005) as well as in seaweed production. Various solutions have been proposed for

the development of alternative strategies in disease control such as anti-bacterial and anti-quorum sensing (QS). Quorum sensing (QS) is the communication among bacteria for the expression of virulence factor which possess in most of aquatic pathogens such as *Aeromonas hydrophila*, *Vibrio campbellii* (Defoirdt et al., 2004), *V. anguillarum* (Milton et al., 1997), *V. parahaemolyticus* (McCarter, 1998) and *Aeromonas salmonicida* (Swift et al., 1997).

In pursuance of combating aquaculture diseases, anti-bacterial compounds have been used widely. However, anti-bacterial compounds have been criticized for the spread of antibiotic resistant bacteria in aquaculture environment. Thus applications of anti-QS compounds could be an alternative solution to antibiotics against aquaculture diseases. The pathogenicity of bacterial strain is depending to the QS mechanism, hence the compound or organism that able to interrupt the QS mechanism will suppress the virulence gene expression of pathogenic bacterial strain. Compound extracted from seaweed has been suggested as another alternative to the antibiotic as a study found that, furanone extracted from red seaweed, *Delisea pulchra* inhibited QS mechanism in *Vibrio harveyi* strain (Manefield et al., 1999). A study by Romero et al. (2010) also found that the bacterial strain isolated from seaweed able to interrupt the QS activity. This showed that the organism or compound that can degrade the QS molecule without the growth interference could be a potential biocontrol agent in aquaculture.

1.2 Problem Statement

Farmers used various types of chemical fertilizers to promote the growth of seaweed hence, increase the production of seaweed. These fertilizers sooner or later will harm the aquaculture environment as well as to the consumers. This showed that there is a need to find a new solution to the chemical fertilizers. Given that there are some studies found that seaweed associated bacteria able to promote the growth and development of seaweed, thus, in this study we focused on the growth promoting properties of the seaweed associated bacteria.

To our knowledge, the information on the symbiotic interaction between *G. changii* and associated bacteria especially on growth and defense mechanism are limited. There is no study describing the beneficial effects of associated bacteria toward *G. changii* especially on the growth promoting properties. Furthermore, anti-QS activity from both *G. changii* and associated bacteria were also investigated. Therefore, this study was conducted to achieve the following objectives:

1. To determine anti-quorum sensing and anti-biofilm activities from *Gracilaria changii* extract
2. To evaluate bacteria isolated from *Gracilaria changii* with anti-pathogenic activities.
3. To study the interaction between *Gracilaria changii* and the associated bacteria for growth promoting activities.

REFERENCES

- Ahmad, S. I. (2006). The seaweed industry in Sabah East Malaysia. *Jati Journal*, 28(11).
- Ali, B., & Hasnain, S. (2007). Potential of bacterial indoleacetic acid to induce adventitious shoots in plant tissue culture. *Letters in Applied Microbiology*, 45(2) 128–133. Doi:10.1111/j.1472-765X.2007.02158.x
- Alik, D.L. (2014). Anti-pathogenic activities from red seaweed (*Gracilaria changii*) and its associated bacteria. *Final Year Project*, pp: 25-28.
- Adonizio, A. L., Downum, K., Bennett, B. C. & Mathee, K. (2006). Anti-quorum sensing activity of medicinal plants in southern Florida. *Journal of Ethnopharmacology*, 105(3), 427-435.
- Anantharaman, P., Karthikaidevi, G., Manivannan, K., Thirumaran, G. & Balasubramanian, T. (2010). Mineral composition of marine macroalgae from Mandapam Coastal Regions; South Coast of India. *Journal of Recent Research in Science and Technology*, 2(10), 66-71.
- Anderson, R. A. (2005). *Algal Culturing Techniques, 1st Edition*. *Journal of Chemical Information and Modeling*, 53(9), 1-596. Doi:10.1017/CBO9781107415324.004
- Andrews, J.H. & Harris, R.F. (2000). The ecology and biogeography of microorganisms on plant surfaces. *Annual Review of Phytopathology*, 38, 145–180.
- Armstrong, E., Yan, L., Boyd, K.G., Wright, P.C., & Burgess, J.G. (2001). The symbiotic role of marine microbes on living surfaces. *Hydrobiologia*, 461, 37-40.
- Ashen, J.B. & Goff, L.J. (2000). Molecular and ecological evidence for species specificity and coevolution in a group of marine algal-bacteria symbioses. *Applied and Environmental Microbiology*, 66, 3024-3030.
- Austin, B., & Austin, D.A. (2007). *Bacterial fish pathogens: disease of farmed and wild fish*. Chichester: Praxis Publishing Company.
- Austin, B., Stobie, M., Robertson, P.A.W., Glass, G.H., Stark, J.R., & Mudarris, M. (1993). *Vibrio alginolyticus*: the cause of gill disease leading to progressive low-level mortalities among juvenile turbot, *Scophthalmus maximus* L., in a Scottish aquarium. *Journal of Fish Diseases*, 16, 277-280
- Balakrishnan, T., Sundaramanickam, A., Veerappan, N., & Sivaperumal, T. (2013). Screening of antibacterial compound from *Cymodocea serrulata* seagrass root extract against human urinary tract infecting pathogens. *Inventi Rapid: Pharm Biotech and Microbio*, 2013(3), 1-4.
- Balasubramanian, V. Palanichamy, S. & Rajaram, R. (2011). Effects of certain seaweed extracts on the primary biofilm forming bacteria. *Journal of the Marine Biological Association of India*, 53 (1), 94-100.
- Bansemir, A., Blume, M., Schröder, S., & Lindequist, U. (2006). Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture*, 252(1), 79–84. Doi:10.1016/j.aquaculture.2005.11.051

- Billot C, Boury S, Benet H, & Kloareg, B. (1999). Development of RAPD markers for parentage analysis in *Laminaria digitata*. *Botanica Marina*, 42, 307–314.
- Bondad-Reantaso, M. G., Subasinghe, R. P., Arthur, J. R., Ogawa, K., Chinabut, S., Adlard, R., & Shariff, M. (2005). Disease and health management in Asian aquaculture. *Veterinary Parasitology*, 132(3-4), 249–272. Doi:10.1016/j.vetpar.2005.07.005
- Bouza, N., Caujapé-Castells, J., González-Pérez, M.A. & Sosa, P.A. (2006). Genetic structure of natural populations in the red algae *Gelidium canariense* (Gelidiales, Rhodophyta) investigated by random amplified polymorphic DNA (RAPD) Markers. *Journal of Phycology*, 42, 304–311.
- Boyd, Kenneth, G., David, R.A. & Burgess, J.G. (1999). Antibacterial and repellent activities of marine bacteria associated with algal surface. *Journal of Bioadhesion and Biofilm Research*, 4, 200-210.
- Brackman, G., Hillaert, U., Van Calenbergh, S., Nelis, H. J., & Coenye, T. (2009). Use of quorum sensing inhibitors to interfere with biofilm formation and development in *Burkholderia multivorans* and *Burkholderia cenocepacia*. *Research in Microbiology*, 160(2), 144–151. Doi :10.1016/j.resmic.2008.12.003
- Brinkhoff, T., Giebel, H. A., & Simon, M. (2008) Diversity, ecology, and genomics of the Roseobacter clade: a short overview. *Archives Microbiology*, 189, 531–539.
- Buschmann, A. H., Hernandez-Gonzalez, M. C. & Varela, D. A. (2008). Seaweed future cultivation in Chile: perspectives and challenges. *International Journal of Environment and Pollution*, 33,432-456.
- Burgess, J.G., Jordan, E.M., Bregu, M., Mearns-Spragg, A. & Boyd, K. (1999). Microbial antagonism: a neglected avenue of natural products research. *Journal of Biotechnology*, 70(1-3), 27-32.
- Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology*, 8, 1137–1144.
- Chan, P. T., Matanjun, P., Yasir, S. M., & Tan, T. S. (2015). Antioxidant activities and polyphenolics of various solvent extracts of red seaweed, *Gracilaria changii*. *Journal of Applied Phycology*, 27(6), 2377–2386. Doi :10.1007/s10811-014-0493-1
- Cheong, C. X., Ho, C. L. & Phang, S. M. (2006). Trends in seaweed research. *TRENDS in Plants Science*, 11(4), 165-166.
- Chu, W. L., Norazmi, M., & Phang, S. M., (2003). Fatty-acid composition of some Malaysian seaweeds. *Malaysian Journal of Science*, 22(2), 21– 27.
- Clements, K. D. (1997). Fermentation and gastrointestinal microorganisms in fishes. in *Gastrointestinal Microbiology*, (pp. 156–198). Springer.
- Csikkle, S. A., Maria, B. & Gerald, B. (1999). Determination of elements in algae by different atomic spectroscopic methods. *Journal of Microchemical*, 67, 39-42.

- Cushnie, T. T., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, 44(5), 377-386.
- Da Gama, B., Carvalho, A., Weidner, K., Soares, A., Coutinho, R., Fleury, B., Teixeira, V., & Pereira, R. (2008). Antifouling activity of natural products from Brazilian seaweeds. *Botany*, 51, 191–201.
- Daud, A., Gallo, A. & Sánchez Riera, A. (2005). Antimicrobial properties of *Phrygilanthus acutifolius*. *Journal of Ethnopharmacology*, 99, 193-197.
- Dawczynski, C., Schubert, R., & Jahreis, G. (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry*, 103(3), 891-899.
- Deep, A., Chaudhary, U., & Gupta, V. (2011). Quorum sensing and bacterial pathogenicity: from molecules to disease. *Journal of Laboratory Physicians*, 3(1), 4–11. Doi: 10.4103/0974-2727.78553
- Defoirdt, T., Boon, N., Bossier, P. & Verstraete, W. (2004). Disruption of bacterial quorum sensing: an unexplored strategy to fight infections in aquaculture. *Journal of Aquaculture*, 240(1-4), 69–88.
- Defoirdt, T., Thanh, L. D., Van Delsen, B., De Schryver, P., Sorgeloos, P., Boon, N., & Bossier, P. (2011). N-acylhomoserine lactone-degrading *Bacillus* strains isolated from aquaculture animals. *Aquaculture*, 311(1-4), 258–260.
- De Nys, R., Steinberg, P. D., Willemsen, P., Dworjanyn, S. A., Gabelish, C. L., & King, R. Y. (1995). Broad spectrum effects of secondary metabolites from red algae *Delisea pulchra* in antifouling assays. *Biofouling*, 8(4), 259–271.
- Dobretsov, S., Teplitski, M., & Paul, V. (2009). Mini-Review: quorum sensing in the marine environment and its relationship to biofouling. *Biofouling*, 25, 413–427.
- DoF. Department of Fisheries Malaysia. (2012). Retrieved 5 July, 2017, from <http://www.dof.gov.my>.
- Dong, Y. H., Xu, J. L., Li, X. Z., & Zhang, L. H., (2000). An enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proceedings of National Academy of Sciences*, 97, 3526–3531.
- Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., Galindo, H. M., Grebmeier, J. M., Hollowed, A. B., Knowlton, N., Polovina, J., Rabalais, N. N., Sydeman, W. J & Talley, L. D. (2012). Climate change impacts on marine ecosystems. *Marine Science*, 4, 11-37.
- Dutcher, J.A. & Kapraun, D.F. (1994). Random amplified polymorphic DNA (RAPD) identification of genetic variation in three species of *Porphyra* (Bangiales, Rhodophyta). *Journal of Applied Phycology*, 6, 267–273.

- Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., & Thomas, T. (2013). The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiology Reviews*, 37(3), 462-476.
- El Bour, M., Ali, A. I., & Ktari, L. (2013). Seaweeds Epibionts : Biodiversity and Potential Bioactivities. In: Mendez-Vilas, A. (Ed.), *Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education. FORMATEX 24, 62* (38), 1298-1306.
- Elsie, B., Sudha, P., & Dhanarajan, M. (2010). N vitro studies on antimicrobial activity and phytochemical analysis of marine algae (seaweeds) *Sargassum witti* and *Gracilaria edulis* using three different solvents extracts. *Theecoscan.in*, 4(4), 343–345. Retrieved from [http://www.theecoscan.in/Journal_PDF/4416- B. HEBSIBAH ELSIE.pdf](http://www.theecoscan.in/Journal_PDF/4416-B_HEBSIBAH_ELSIE.pdf)
- Fernandes, N., Steinberg, P., Rusch, D., Kjelleberg, S., & Thomas, T. (2012). Community structure and functional gene profile of bacteria on healthy and diseased thalli of the red seaweed *Delisea pulchra*. *PloS one*, 7(12), e50854.
- Food and Agriculture Organization of the United Nations (FAO) (2014). *FAO Statistical Yearbook 2014- Asia and the Pacific Food and Agriculture*. Doi :<http://www.fao.org/docrep/018/i3107e/i3107e.PDF>
- Food and Agriculture Organization of the United Nations (FAO) (1996). *Regional Study and Workshop on the Taxonomy, Ecology and Processing of Economically Important Red Seaweeds. Food and Agriculture Organization of the United Nations Network of Aquaculture Centres in Asia-Pacific*. Bangkok, Thailand.
- Food and Agriculture Organization of the United Nations (FAO) (2010). *State of World Fisheries and Aquaculture*. FAO Fisheries Department . Food and Agriculture Organization of the United Nations, Rome, Italy.
- Food and Agriculture Organization of the United Nations (FAO) (2012). *The State of World Fisheries and Aquaculture 2012*. Food and Agriculture Organization of the United Nations, Rome, pp 209.
- Food and Agriculture Organization of the United Nations (FAO) (2015). *Training Manual on Gracilaria Culture and Seaweed Processing in China: Chapter I biology of Gracilaria*. Retrieved from <http://www.fao.org/docrep/field/003/AB730E/AB730E01.htm>
- Fouladynezhad, N., Afsah-Hejri, L., Rukayadi, Y., Abdulkarim, S. M., Marian, M. N. & Son, R (2013). Assessing biofilm formation by *Listeria monocytogenes*. *International Food Research Journal*, 20(2), 987-990.
- Fouz B., Larsen J.L., & Amaro C. (2006). *Vibrio vulnificus* serovar a: an emerging pathogen in European anguilliculture. *Journal of Fish Diseases*, 29, 285-291.
- Gan, M.H., Siti Aisyah, A., Nur Wahidah, A., Amyra Suryatie, K. & Noraien, M.P. (2011). Diversity of seaweed in the vicinity of Johor: with emphasis on the east coast peninsular Malaysia expedition II 2006. *Proceedings of Universiti Malaysia*

Terengganu International Annual Symposium, Terengganu, Malaysia, July 11-13, 2011, 429-433

- Givskov, M., de Nys, R., & Manefield, M., et al. (1996). Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. *Journal of Bacteriology*, *178*, 6618–6622.
- Goecke, F., Labes, A., Wiese, J. & Imhoff, J.F. (2010). Chemical interaction between marine macroalgae and bacteria. *Marine Ecology Progress Series*, *409*, 267-300.
- González, F., & Silva, M. (2001). Biodiversidad química de maroalgas marinas. In: Alveal K. & T. Antezana (eds). *Sustentabilidad de la biodiversidad, un problema actual. Bases científicotécnicas, teorizaciones y proyecciones*. pp: 415-496. Translated in English.
- González, M., Rolando, M., Candia, A., Gómez, P., & Cisternas, M., (1996). Organellar DNA restriction fragment length polymorphism (RFLP) and nuclear random amplified polymorphic DNA (RAPD) analyses of morphotypes of *Gracilaria* (*Gracilariales*, Rhodophyta) from Chile. *Hydrobiologia*, *326/327*, 229–234.
- Gordon, S.A., & Weber, R.P. (1951). Colorimetric estimation of indolacetic acid. *Plant Physiology*, *26*, 192–195.
- Guiry, M.D. & Guiry, G.M. (2017). *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on 17 July 2017.
- Hellio, C., De, La, Broise, D., Dufosse, L., Le, Gal, Y., & Bourgoignon, N., (2001). Inhibition of marine bacteria by extracts of macroalgae: potential use for environmentally friendly antifouling paints. *Marine Environmental Research*, *52*, 231–247.
- Ho, C.L., Phang, S.M., & Tikki, P., (1995). Application of polymerase chain reaction (PCR) using random amplified polymorphic DNA (RAPD) primers in the molecular identification of selected *Sargassum* species (Phaeophyta, Fucales). *European Journal of Phycology*, *30*, 273–280.
- Hollants, J., Leliaert, F., De Clerck, O., & Willems, A. (2013). What we can learn from sushi: A review on seaweed-bacterial associations. *FEMS Microbiology Ecology*, *83*(1), 1–16. Doi :10.1111/j.1574-6941.2012.01446.x
- Hollants, J., Leroux, O., Leliaert, F., Decleyre, H., De-Clerck, O. & Willems, A. (2011). Who is in there? Exploration of endophytic bacteria within the siphonous green seaweed *Bryopsis* (Bryopsidales, Chlorophyta). *PLoS ONE*, *6*, 58.
- Hollants, J (2012). Endophytic bacteria within the green siphonous seaweed *Bryopsis*: exploration of a partnership. PhD thesis, Ghent University, Ghent, Belgium.
- Huggett, M.J., Williamson, J.E., De Nys, R., Kjelleberg, S., & Steinberg, P.D. (2006). Larval settlement of the common Australia sea urchin *Heliocidaris erythrogramma* in response to bacteria from the surface of coralline algae. *Oecologia*, *149*, 604-619.

- Huh, M.K., Lee, B.K., & Lee, H.Y., (2006). Genetic diversity and phylogenetic relationships in five *Porphyra* species revealed by RAPD analysis. *Protistology*, 4, 245–250.
- Hu Y., Yu G., Zhao X., Wang Y., Sun X., Jiao G., Zhao X., & Chai W. (2012). Structural characterization of natural ideal sulfated agarose from red alga *Gloiopeltis furcata*. *Carbohydrate Polymers*, 89(3), 883-889.
- Isnansetyo, A. & Kamei, Y. (2003). MC21-A, a bactericidal antibiotic produced by a new marine bacterium, *Pseudoaltermononas phenolica* sp. nov. O-BC30T, against Methicillin-Resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, 47, 480-488.
- Issac, A. S. V. P., Palani, A., Khadar, S. M., Shunmugiah, K. P. & Arumugam, V. R. (2012). Antibiofilm and quorum sensing inhibitory potential of *Cuminum cyminum* and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens. *Food Research International*, 45, 85-92.
- Jha, B., Kavita, K., Westphal, J., Hartmann, A., & Schmitt-Kopplin, P. (2013). Quorum sensing inhibition by *Asparagopsis taxiformis*, a marine macro alga: separation of the compound that interrupts bacterial communication. *Marine Drugs*, 11(1), 253–265. Doi :10.3390/md11010253
- Johannes Reinke (1903). Symbiose von *Volvox* und *Azotobacter*. *Ber. Deutsch. Bot. gesellsch*, 21, 481-483. Translated in English.
- Joint, I., Tait, K., & Wheeler, G. (2007). Cross-kingdom signalling: exploitation of bacterial quorum sensing molecules by the green seaweed *Ulva*. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 362(1483), 1223-1233. Doi :10.1098/rstb.2007.2047
- Kain, J.M. (1991). Cultivation of Attached Seaweeds. In Guiry, M.D. and Bluden, G. 1992. *Seaweed Resources in Europe: Uses and Potential*. Jon Wiley and Sons, Chichester ISBN 0-471-92947-6.
- Kanji, H., Takahiro, Y. Keisuke, M. & Keiji, I. (1979). Distribution of Quaternary Ammonium Bases in Seven Species of Marine Algae. *Journal of Faculty of Applied Biological Science (Hiroshima University)*, 18, 65-73.
- Kalhoefter, D., Thole, S., Voget, S., Lehmann, R., Liesegang, H., Wollher, A., Daniel, R., Simon, M., & Brinkhoff, T. (2011). Comparative genome analysis and genome-guided physiological analysis of *Roseobacter litoralis*. *BMC Genomics*, 12, 324.
- Kalia, V. C. (2013). Quorum sensing inhibitors: an overview. *Biotechnology Advances*, 31(2), 224-245.
- Kalia, V.C., & Kumar, P. (2015). Potential applications of quorum sensing inhibitors in diverse field. In: Kalia VC (ed) quorum sensing vs quorum quenching: a battle with no end in sight, pp 359-370. Springer India.
- Kaliaperumal, N., Kalimuthu, S., & Ramalingam, J. R. (1989). Agar, align and mannitol from some seaweeds of Lakshadweep. *Journal of the Marine Biological Association of India*, 31(1&2), 303-305.

- Kannan, R.R.R., Arumugam, R. & Anantharaman, P. (2012). Chemical composition and antibacterial activity of Indian seagrasses against urinary tract pathogens. *Food Chemistry*, 135, 2470-2473.
- Kannapiran, E. & Nithyanandan, M. (2002). Antibacterial activity of different fractions of extracts from Palk Bay seaweeds. *Seaweeds Research and Utilization*, 24(1),177-181
- Kayalvizhi, K., Subramanian, V., Anantharaman, P. & Kathiresan, K. (2012). Antimicrobial activity of seaweeds from the gulf of Mannar. *International Journal of Pharmaceutical Applications*, 3, 306-314.
- Keshtacher-Liebson, E., Hadar, Y., & Chen, Y. (1995). Oligotrophic bacteria enhance algal growth under iron-deficient conditions. *Applied and Environmental Microbiology*, 61(6), 2439–2441.
- Kolanjinathan, K. & Saranraj, P. (2014). Pharmacological efficacy of marine seaweed *Gracilaria edulis* extracts against clinical pathogens. *Global Journal of Pharmacology*, 8(2), 268-274.
- Kulshreshtha, G., Rathgeber, B., Stratton, G., Thomas, N., Evans, F., Critchley, A., Hafting, J., & Prithiviraj, B. (2014). Feed supplementation with red seaweeds, *Chondrus crispus* and *Sarcoditheca gaudichaudii*, affects performance, egg quality, and gut microbiota of layer hens. *Poultry Science*, 93, 2991–3001.
- Lakshmanaperumalsamy, P. & Purushothaman, A. (1982). Heterotrophic bacteria associated with seaweed. *Proceedings of the Indian Academy of Science (Plant Sci.)*, 91, 487-493.
- Lam, C., & Harder, T. (2007). Marine macroalgae effect abundance and community richness of bacterioplankton in close proximity. *Journal of Phycology*, 43, 874–881.
- Laycock, R. A. (1974). The detrital food chain based on seaweeds. I. Bacteria associated with the surface of Laminaria fronds. *Marine Biology*, 25(3), 223–231. Doi :10.1007/BF00394968
- Lewmanomont, K. & Ogawa, H. (1995). Common seaweed and seagrasses of Thailand. Faculty of Fisheries, Kasetsart University, Bangkok, 163pp.
- Lei, Q., Yin-Geng, W., Zheng, Z & Shao-Li, L (2006). The first report on fin rot disease of cultured turbot *Scophthalmus maximus* in China. *Journal of Aquatic Animal Health*, 18, 83-89
- Lim, P.E., & Phang, S.M. (2004). *Gracilaria* species (Gracilariaceae, Rhodohyta) of Malaysia including two new records. *Malaysia Journal of Science*, 23, 71-80.
- Lim, P. E., Thong, K. L., & Phang, S. M., (2001). Molecular differentiation of two morphological variants of *Gracilaria salicornia*. *Journal of applied Phycology* 13, 335–342.
- Lindequist, U. & Schweder, T. (2001). Marine Biotechnology. In: Rehm, H.J., Reed, G. (Eds.), *Biotechnology*. 10, 441-484.

- Maithili, S. S., Thangadurai, G., & Ramanathan, G. (2014). Isolation of Secondary Metabolites from Marine Algal bacterial Population against Foot Ulcer Associated Pathogens. *International Journal of Current Microbiology and Applied Sciences*, 3(3), 196–205.
- Manefield, M., de Nys, R., Kumar, N., Read, R., Givskov, M., Steinberg, P. D. & Kjelleberg, S. (1999). Evidence that halogenated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL) mediated gene expression by displacing the AHL signal from its receptor protein. *Microbiology*, 145, 283–91.
- Marshall, K., Joint, I., Callow, M.E. and Callow, J.A. (2006). Effect of marine bacterial isolates on the growth and morphology of axenic plantlets of the green alga *Ulva linza*. *Microbial Ecology*, 52,302-310.
- Martinez, E.A., Destombe, C., Quillet, M.C., & Valero, M. (1999). Identification of random amplified polymorphic DNA (RAPD) markers highly linked to sex determination in the red alga *Gracilaria gracilis*. *Molecular Ecology*, 8, 1533–1538.
- Maruyama, A., Maeda, M. & Simidu, U. (1986) Occurrence of plant hormone (cytokinin)-producing bacteria in the sea. *Journal of Applied Bacteriology*, 61, 569-574.
- Maruyama, A., Yamaguchi, I., Maeda, M., & Shimizu, U. (1988). Evidence of cytokinin production by a marine bacterium and its taxonomic characteristics. *Canada Journal of Microbiology*, 34, 829–833. Doi: 10.1139/m88-142
- Matsuo, Y., Suzuki, M., Kasai, H., Shizuri, Y. & Harayama, S. (2003). Isolation and phylogenetic characterization of bacteria capable of inducing differentiation in the green alga *Monostroma oxyspermum*. *Environmental Microbiology*, 5, 25–35.
- Matsuo, Y., Imagawa, H., Nishizawa, M., & Shizuri, Y. (2005). Isolation of an algal morphogenesis inducer from a marine bacterium. *Science*. 307, 1598.
- Maximilien, R., de Nys, R., Holmstrom, C., Gram, L., Givskov, M., & Crass, K. (1998). Chemical mediation of bacterial surface colonisation by secondary metabolites from the red alga *Delisea pulchra*. *Aquatic Microbiology Ecology*.
- Mazarrasa, I., Olsen, Y.S., Mayol, E., Marbà, N., & Duarte, C.M. (2014). Global unbalance in seaweed production, research effort and biotechnology markets. *Biotechnology Advances*, 32, 1028-1036.
- McCarter, L. L. (1998). OpaR, a homolog of *Vibrio harveyi* LuxR, controls opacity of *Vibrio parahaemolyticus*. *Journal of Bacteriology*, 180(12), 3166–3173.
- McClean, K.H., Michael, K.W., Leigh, F., Adrian, T., Siri Ram, C., Miguel, C. & Mavis, D. (1997). Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Journal of Microbiology*, 143(12), 3703-3711.
- Milton, D.L., Hardman, A., Camara, M., Chhabra, S.R., Bycroft, B.W., Stewart, G.S., & Williams, P(1997). Quorum sensing in *Vibrio anguillarum*: characterization of the vanI/vanR locus and identification of the autoinducer N-(3-Oxodecanoyl)-L-Homoserine lactone. *Journal of Bacteriology*, 179, 3004–3012.

- Mohamed, S., Hashim, S.N., & Rahman, H.A. (2012). Seaweeds: a sustainable functional food for complementary and alternative therapy. *Trends Food Science and Technology*, 23, 83–96.
- Mohammad, R., Ahmad, M.F., Noh, N.F.M, Saari, N.A. & Othman, A.M. (2013). Viability and competitiveness of seaweed industry in Malaysia. *Economic and Technology Management Review*, 8, 1-11.
- Mouritsen, O. G., Dawczynski, C., Duelund, L., Jahreis, G., Vetter, W., & Schröder, M. (2013). On the human consumption of the red seaweed dulse (*Palmaria palmata* (L.) Weber & Mohr). *Journal of Applied Phycology*, 25(6), 1777-1791.
- Muta Harah, Z., Chia, W.S., Japak Sidik, B., Arshad., A. & Ogawa, H. (2007). Macroalgae Diversity and Life Forms of Inter-tidal Rocky Shores. *Marine Research in Indonesia*, 163-168.
- Nakanishi, K., Nishijima, M., Nomoto, A.M., Yamazaki, A., & Saga, N. (1999). Requisite morphologic interaction for attachment between *Ulva pertusa* (Chlorophyta) and symbiotic bacteria. *Marine Biotechnology*, 1, 107-111.
- Natrah, F. M. I., (2011). Role of bacteria quorum sensing and micro-algae in fish and crustacean larviculture. PhD thesis, Ghent University, Ghent, Belgium.
- Natrah, F. M. I., Defoirdt, T., Sorgeloos, P., & Bossier, P. (2011). Disruption of Bacterial Cell-to-Cell Communication by Marine Organisms and its Relevance to Aquaculture. *Marine Biotechnology*, 13(2), 109–126.
- Nedumaran, T., & Arulbalachandran, D. (2015). Seaweeds: A promising source for sustainable development. In : P. Thangavel and G. Sridevi (eds.), *Environmental Sustainability*, (pp. 65-85). Springer India. Doi : 10.1007/978-81-322-2056-5_4
- Nelson, K. H., & Hastings, J. W (1979). Bacterial bioluminescence: its control and ecological significance. *Microbiological Reviews*, 43, 496-518.
- Newman, D.J., Cragg, G.M., & Snader, K.M. (2003). Natural product as sources of new drugs over the period 1981-2002. *Journal of Natural Products*, 66, 1022-1037.
- Nielsen M.E., Hoi L, Schmidt A.S., Qian D., Shimada T., Shen J.Y., & Larsen J.L. (2001). Is *Aeromonas hydrophilla* the dominant motile *Aeromonas* species that cause disease outbreaks in aquaculture production in the Zhejiang Province of China?. *Diseases of Aquatic Organisms*, 46, 23-29.
- Norashikin, A., Muta Harah., Z. & Japar Sidik, B. (2013). Intertidal seaweeds and their multi-life forms. *Journal of Fisheries and Aquatic Science*, 8(3), 452-461.
- Norizan, M.A. (1999). Kajian Taksonomi *Gracilaria* (Rhodophyta) dan Pengkulturannya. *Universiti Kebangsaan Malaysia*.
- Norziah, M.H. & Ching, C.Y. (2000). Nutritional composition of edible seaweed *Gracilaria changii*. *Food chemistry*, 68, 69-76.

- Nyholm, S. V., & McFall-Ngai, M. J. (1998). Sampling the light-organ microenvironment of *Euprymna scolopes*: description of a population of host cells in association with the bacterial symbiont *Vibrio fischeri*. *The Biological Bulletin*, 195,89-97
- Nylund, G. M., Persson, F., Lindegarth, M., Cervin, G., Hermansson, M., & Pavia, H. (2010). The red alga *Bonnemaisonia asparagoides* regulates epiphytic bacterial abundance and community composition by chemical defence. *FEMS Microbiology Ecology*, 71(1), 84–93. Doi :10.1111/j.1574-6941.2009.00791.x
- Park, J.W., Cho, Y.C., Nam, B.H., Jin, H.Y., Sohn, C.H., & Hong, Y.K. (1998). RAPD identification of genetic variation in seaweeds *Hizikia fusiformis* (Fucales, Phaeophyta). *Journal of Marine Biotechnology*, 6:62–64
- Palanichamy, S. (2011). Effects of certain seaweed extracts on the primary bio-film forming bacteria. *Journal of Marine Biological Association of India*, 53 (1), 94 - 100.
- Park, M., Km, C., Yang, J., Lee, H., ShinW, Kim, S., & Sa, T. (2005). Isolation and characterization of diazotrophic growth promotion bacteria from rhizosphere of agricultural crop of Korea. *Microbiological Research*, 160, 127–133.
- Patwary MU, MacKay RM, Van der Meer JP (Food and Agriculture Organization of the United Nations (FAO). (2014). *FAO Statistical Yearbook 2014- Asia and the Pacific Food and Agriculture*. Doi :<http://www.fao.org/docrep/018/i3107e/i3107e.PDF>
- Patwary M.U., MacKay, R.M., & Van der Meer, J.P. (1993). Revealing genetic markers in *Gelidium vagum* (Rhodophyta) through the random amplified polymorphic DNA (RAPD) technique. *Journal of Phycology*, 29, 216–222.
- Performance Management and Delivery Unit (PEMANDU). (2010). Agriculture. *ETP Handbook*, 513–550. Doi :10.3233/WOR-141920
- Petrini, L.E., Petrini, O., & Laflamme, G. (1989). Recovery of endophytes of *Abies balsamea* from needles and galls of *Paradiplosis tumifex*. *Phytoprotection*, 70, 97–103.
- Phang, S. M., 1984. Seaweed Resources of Malaysia. *Wallaceana*, 33, 3–8.
- Phang, S.M. (2010). Potential Products from Tropical Algae and Seaweeds, especially with Reference to Malaysia. *Malaysian Journal of Science*, 29(2), 160-166.
- Phang, S.M. (2006). Seaweed resources in Malaysia: current status and future prospects. *Aquatic Ecosystem Health and Management*, 9, 185–202.
- Phang, S.M., Shaharuddin, S., Noraishah, H., & Sasekumar, A. (1996). Studies on *Gracilaria changii* (Gracilariaceae, Rhodohyta) from Malaysian mangroves. *Hydrobiologia*, 326/327, 347-352.
- Phang, S.M., & Lewmanomont, K. (2001) *Gracilaria changii* (B. M. Xia & I. A. Abbott) I. A. Abbott, C. F. Chang & B. M. Xia. In: Reine P, Trono GC Jr (eds) Plant resources of South-East Asia, no. 15(1). Cryptogams: algae. Backhuys, Leiden, 178–180.

- Polifrone, M., Masi, F. D., & Gargiulo, G. M. (2006). Alternative pathways in the life history of *Gracilaria gracilis* (Gracilariales, Rhodophyta) from north-eastern Sicily (Italy). *Aquaculture*, 1003-1013.
- Prabhakaran, S., Rajaram, R., Balasubramanian, V., & Mathivanan, K. (2012). Antifouling potentials of extracts from seaweeds, seagrasses and mangroves against primary biofilm forming bacteria. *Asian Pacific Journal of Tropical Biomedicine*, 2(1 SUPPL.). Doi : 10.1016/S2221-1691(12)60181-6
- Prasad, V. G. N. V., Swamy, P. L., Rao, T. S., & Rao, G. S. (2013). Antibacterial synergy between oxytetracycline and selected polyphenols against bacterial fish pathogens. *International Journal of Veterinary Science*, 2(2), 71-74.
- Provasoli, L. (1958). Effect of plant hormones on *Ulva*. *The Biological Bulletin*, 114, 375-384.
- Rafael, L., Claire, M. M. G. & Celine, R. (2015). Seaweed cultivation: potential and challenges of crop domestication at an unprecedented pace. *New Phytologist*, 206(2), 486-492.
- Rao, P.S.P. (1991). Biological investigation of Indian marine algae and screening of some green, red and brown seaweeds for their antimicrobial activity. *Seaweed Research and Utilization*, 14(1),37-43.
- Renn, D.W. (1992). In *Marine Biotechnology: Pharmaceutical and Bioactive Natural Products*, 1,181-196.
- Romero, M., Cuadrado, A.B.M., Rivada, A.R., Cabello, A.M. & Otero, A. (2010). Quorum quenching in cultivable bacteria from dense marine coastal microbial communities. *FEMS Microbiology Ecology*, 75, 205-217.
- Santelices, B., & Doty, M. S. (1989). A review of *Gracilaria* farming. *Aquaculture*. Doi :10.1016/0044-8486(89)90026-4
- Sasidharan, S., Darah, I., & Jain, K. (2009) : Screening antimicrobial activity of various extracts of *Gracilaria changii*. *Pharmaceutical Biology*, 47(1), 72-76.
- Sasidharan S, Darah, I., & Jain, K. (2011). In vitro and in situ antiyeast activity of *Gracilaria changii* methanol extract against *Candida albicans*. *European Review for Medical and Pharmacological Sciences*, 15(9), 1020–1026.
- Sfanos, K., Harmody, D., Dang, P., Ledger, A., Pomponi, S., McCarthy, P., & Lopez, J. (2005). A molecular systematic survey of cultured microbial associates of deep-water marine invertebrates. *Systematic and applied microbiology*, 28(3), 242-264.
- Shu, M.H., Appleton, D., Zandi, K., & AbuBakar, S. (2013). Anti-inflammatory, gastroprotective and antiulcerogenic effects of red algae *Gracilaria changii* (Gracilariales, Rhodophyta) extract. *BMC Complementary and Alternative Medicine*, 13,62.
- Sifri, C. D. (2008). Healthcare epidemiology: quorum sensing: bacteria talk sense. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of*

America, 47(8), 1070–1076. Doi :10.1086/592072

- Sim, M. C., Lim, P. E., Gan, S. Y., & Phang, S. M. (2007). Identification of random amplified polymorphic DNA (RAPD) marker for differentiating male from female and sporophytic thalli of *Gracilaria changii* (Rhodophyta). *Journal of Applied Phycology*, 19(6), 763–769. Doi :10.1007/s10811-007-9224-1
- Singh, R.P. (2013). Isolation and characteratization of exopolysaccharides from seaweed associated bacteria *Bacillus licheniformis*. *Carbohydrate Polymers*, 84, 1019-1026.
- Singh, R. P., & Reddy, C. R. K. (2014). Seaweed-microbial interactions: Key functions of seaweed-associated bacteria. *FEMS Microbiology Ecology*, 88(2), 213–230. <http://doi.org/10.1111/1574-6941.12297>
- Singh, R. P., Baghel, R. S., Chennur, R. R., & Jha, B. (2015). Plant-Microbe Interaction Effect of quorum sensing signals produced by seaweed-associated bacteria on carpospore liberation from *Gracilaria dura* Article type : Received on : Accepted on : Frontiers website link : Citation : Effect of quorum sensing signal, 6(March), 1–13. Doi :10.3389/fpls.2015.00117
- Singh, R. P., Bijo, J., Baghel, R. S., Reddy, C. R. K., & Jha, B. (2011a). Role of bacterial isolates in enhancing the bud induction in the industrially important red alga *Gracilaria dura*. *FEMS Microbiology Ecology*, 76(2), 381–392. Doi :10.1111/j.1574-6941.2011.01057.x
- Singh, R. P., Mantri, V. A., Reddy, C. R. K., & Jha, B. (2011b). Isolation of seaweed-associated bacteria and their morphogenesis-inducing capability in axenic cultures of the green alga *Ulva fasciata*. *Aquatic Biology*, 12(1), 13–21. Doi :10.3354/ab00312
- Singh, R. P., Shukla, M. K., Mishra, A., Reddy, C. R. K., & Jha, B. (2013). Bacterial extracellular polymeric substances and their effect on settlement of zoospore of *Ulva fasciata*. *Colloids and Surfaces B: Biointerfaces*, 103, 223–230. Doi :10.1016/j.colsurfb.2012.10.037
- Smith, J. E. & Williams, S. L. (2007). A Global Review of the Distribution , Taxonomy , and Impacts of Introduced Seaweeds. *Annual Review of Ecology, Evolution, and Systemics*, 38, 327-359.
- Spoerner, M., Wichard, T., Bachhuber, T., Stratmann, J., & Oertel, W. (2012). Growth and thallus morphogenesis of *Ulva mutabilis* (Chlorophyta) depends on a combination of two bacterial species excreting regulatory factors. *Journal of Phycology*, 48, 1433–1447.
- Steinberg, P.D., & Van Altna, I. (1992). Tolerance of marine invertebrate herbivores to brown algal phlorotannins in temperate Australasia. *Ecological Monographs*, 62, 189–222.
- Sturz A.V., Christie, B.R., & Nowak, J. (2000). Bacterial endophytes: potential role in developing sustainable systems of crop production. *Critical Reviews in Plant Sciences*, 19, 1–30.

- Sudheesh, P. S., & Xu, H-S. (2001). Pathogenicity of *Vibrio parahaemolyticus* in tiger prawn *Penaeus monodon* Fabricius: possible role of extracellular proteases. *Aquaculture*, 196, 37-46.
- Susilowati, R. Agus, S. & Ita, W. (2015). Isolation and characterization of bacteria associated with brown algae *Sargassum* spp. from Panjang Island and their antibacterial activities. *Procedia Environmental Sciences*, 23, 240-246.
- Swift, S., Karlyshev, A. V, Fish, L., Durant, E. L., Winson, M. K., Chhabra, S. R. A. M., & Stewart, G. S. A. B. (1997). Quorum Sensing in *Aeromonas hydrophila* and *Aeromonas salmonicida* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N -acylhomoserine lactone signal molecules. *Journal of Bacteriology*, 179(17), 5271–5281.
- Taga, M.E., & Bassler, B.L. (2003). Chemical communication among bacteria. *Proceedings of the National Academy of Sciences of the USA*, 100, 14549– 14554.
- Tatewaki, M, Provasoli, L., & Pinter, I.J. (1983). Morphogenesis of *Monostroma oxysperma* (Kütz.) Doty (Chlorophyceae) in axenic culture, especially in bialgal culture. *Journal Phycology*, 19,404-416.
- Tsavkelova, E.A., Cherdyntseva, T.A., & Netrusov, A.I. (2005). Auxin production by bacteria associated with orchid roots. *Microbiology*, 74, 46–53.
- Ugarte, R. & Santelices, B (1992). Experimental tank cultivation of *Gracilaria* in central Chile. *Aquaculture* 101,7-16.
- Valatka, S., Makinen, A., & Yli-Mattila, T. (2000) Analysis of genetic diversity of *Furcellaria lumbricalis* (Gigartinales, Rhodophyta) in Baltic Sea by RAPD-PCR technique. *Phycologia*, 39, 110–117.
- Vallinayagam, K., Arumugam, R. and Kannan, R.R. (2009). Antibacterial activity of some selected seaweeds from Pudumadam Coastal Regions. *Global Journal of Pharmacology*,3(1),50-52.
- Veselova, M., Kholmeckaya, M., Klein, S., Voronina, E., Lipasova, V., Metlitskaya, A., Mayatskaya, A., Lobanok, E., Khmel, I., & Chernin, L (2003). Production of N-acylhomoserine lactone signal molecules by gram-negative soil-borne and plant-associated bacteria. *Folia Microbiologica*, 48, 794–798.
- Viju, N., Anitha, A., Vini, S. S., Shankar, C. V. S., Satheesh, S., & Punitha, S. M. J. (2014). Antibiofilm activities of extracellular polymeric substances produced by bacterial symbionts of seaweeds. *Indian Journal of Geo-Marine Sciences*, 43(11), 2136–2146.
- Villarreal-Gómez, L. J., Soria-Mercado, I. E., Guerra-Rivas, G., & Ayala-Sánchez, N. E. (2010). Antibacterial and anticancer activity of seaweeds and bacteria associated with their surface. *Revista de Biología Marina Y Oceanografía*. Doi :10.4067/S0718-19572010000200008

- Visick, K. L., & McFall-Ngai, M. J. (2000). An exclusive contract: Specificity in the *Vibrio fischeri*–*Euprymna scolopes* partnership. *Journal of Bacteriology*, *182*, 1779–1787.
- Wagner-Dobler, I., Thiel, V., Eberl, L., Allgaier, M., Bodor, A., Meyer, S., Ebner, S., Hennig, A., Pukall, R., & Schulz, S. (2005). Discovery of complex mixtures of novel long-chain quorum sensing signals in free-living and host-associated marine *Alphaproteobacteria*. *Journal of ChemBiochem*, *6*, 2195–2206.
- Wahl, M., Goecke, F., Labes, A., Dobretsov, S., & Weinberger, F. (2012). The second skin: ecological role of epibiotic biofilms on marine organisms. *Frontiers in microbiology*, *3*.
- Waksman, S.A., Hotchkiss, M., & Carey, C.L. (1933). Marine bacteria and their role in the cycle of life of the sea: II. bacteria concerned with the cycle of nitrogen in the sea. *The Biology Bulletin*, *65*, 137–167.
- Weinberger, F., Friedlander, M., & Gunkel, W. (1994). A bacterial facultative parasite of *Gracilaria conferta*. *Diseases of Aquatic Organisms*, *18*, 135–141.
- Weinberger, F., Hoppe, H.G., & Friedlander, M. (1997). Bacterial induction and inhibition of a fast necrotic response in *Gracilaria conferta* (Rhodophyta). *Journal of Applied Phycology*, *9*, 277–285.
- Whitehead, N. A., Barnard, A. M. L., Slater, H., Simpson, N. J. L., & Salmond, G. P. C. (2001). Quorum-sensing in Gram-negative bacteria. *FEMS Microbiology Reviews*, *25*(4), 365–404.
- Widowati, I., Lubac, D., Puspita, M., & Bourgougnon, N. (2014). Anti-bacterial and antioxidant properties of the red alga *Gracilaria verrucosa* from the north coast of Java, Semarang, Indonesia. *International journal of latest research in Science and Technology*, *3*(3), 179–185.
- Wiese, J., Thiel, V., Nagel, K., Staufenberger, T., & Imhoff, J. F. (2009). Diversity of antibiotic-active bacteria associated with the brown alga *Laminaria saccharina* from the baltic sea. *Marine Biotechnology*, *11*(2), 287–300. Doi :10.1007/s10126-008-9143-4
- Wong, C.L., Gan, S.Y., & Phang, S.M. (2004). Morphological and molecular characterization and differentiation of *Sargassum baccularia* and *S. polycystum* (Phaeophyta). *Journal of Applied Phycology*, *1*, 439–445.
- Wong, P. F., Tan, L. J., Nawi, H., & AbuBakar, S. (2006). Proteomics of the red alga, *Gracilaria changii* (Gracilariales, Rhodophyta). *Journal of Phycology*, *42*(1), 113–120. Doi :10.1111/j.1529-8817.2006.00182.x
- Wu, B., Tseng, C. K., & Xiang, W. (1993). Large-scale cultivation of *Spirulina* in seawater based culture medium. *Botanica Marina*, *36*(2), 99–102.
- Xia BM, & Abbott IA (1987). New species of *Polycarvernosa* Chang and Xia (*Gracilariaceae*, Rhodophyta) from the western Pacific. *Phycologia*, *26*, 405–418.

- Yarish, C., Redmond, S. & Kim, J.K. (2012). *Gracilaria* culture handbook for New England. *Wrack Lines*, 72,6-14.
- Yeong, H. Y., Phang, S. M., Reddy, C. R. K., & Khalid, N. (2014). Production of clonal planting materials from *Gracilaria changii* and *Kappaphycus alvarezii* through tissue culture and culture of *G. changii* explants in airlift photobioreactors. *Journal of Applied Phycology*, 26(2), 729–746. Doi :10.1007/s10811-013-0122-4
- Zhang, X.H., & Austin, B. (2000). Pathogenicity of *Vibrio harveyi* to salmonids. *Journal of Fish Diseases*, 23, 93-102.
- Zheng, L., Han, X., Chen, H., Lin, W., & Yan, X. (2005). Marine bacteria associated with marine macroorganisms: the potential antimicrobial resources. *Annals of Microbiology*, 55(2), 119–124.
- Zhou, Y., Yang, H., Hu, H., Liu, Y., Mao, Y., Zhou, H., ... Zhang, F. (2006). Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. *Aquaculture*, 252(2–4), 264–276. Doi :10.1016/j.aquaculture.2005.06.046
- Zinger, L., Amaral-Zettler, L.A., & Fuhrman, J.A. (2011). Global patterns of bacterial beta diversity in seafloor and seawater ecosystems. *PLoS ONE* 6: e24570.