MARINE MICROBIAL DIVERSITY OF OFF-TERENGGANU COASTAL SEDIMENT IN SOUTH CHINA SEA

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"*Blessed is He in Whose Hand is the dominion, and He is able to do all things, Who has created death and life, that He may test you which of you is best in deed, and He is the All-Mighty, the Oft-Forgiving*."

[Al-Mulk 67:1-2]

This thesis is especially dedicated to my beloved family: Hj. Zahar, Hjh. Marisah, Zairi and Marzarina.

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ABSTRACT

Marine bacteria play a vital role in regulating global biochemical cycle for billions of years, and their function has been widely explored for the past fifty years. Marine bacteria exploration is considered as difficult and precarious, but every finding is fruitful in providing information to generate a better understanding of its purpose in the seawater. Marine bacteria exploration in Malaysia coastline is considered as new with no impactful data to represent the bacteria distribution in Malaysia’s coastline, specifically heading towards the South China Sea. The purpose of this study is to assess bacteria diversity off-Terengganu coast as the foremost marine bacteria abundance screening in these areas. In this study, surface sea sediment that contains a variety of bacteria cells is collected in three random locations with three different depths. The DNA obtained from the cell extraction was identified with Next Generation Sequence method, which specifically targeted 16SrDNA V3-V4 properties to obtain the overall bacterial metagenomic profile. Results showed that off-Terengganu coast, bacteria diversity consisted of 25518 amplicons of 3301 unique OTUs, which signify 27 phyla. The OTU abundance decreased gradually with depth of sediment in the sea. The metagenomic profile revealed two sulphur-degrading bacteria were dominant in the surveyed area. *Sulfurovum* genus dominate overall bacteria community in two locations situated in the northeast area of sampling stations. Conversely, *Pseudoalteromonas* dominated the overall bacterial community in the southeast coastline. The Physical-geochemical analysis revealed that all surveyed areas contained sulphur, oil, grease, gasoline, diesel, and mineral oil, which perhaps are influencing sulphur-degraded bacteria community growth in the surveyed area. There is no concrete evidence to link *Sulfurovum* and *Pseudoalteromonas* as pathogenic bacteria that causes illness to the human. However, there are possibility that the surveyed areas are anthropogenically polluted and further physical-geochemical analysis is required. In conclusion, the research findings suggested the necessity to conduct a broader bacteria diversity research, such as bacterial dispersion scale, and community variation in order to measure an inordinate extent of environmental pollution in the surveyed areas.

ABSTRAK

Bakteria marin memainkan peranan penting dalam mengawal selia kitaran biokimia global sejak berbilion-billion tahun dan fungsi ini telah diterokai secara meluas lima puluh tahun yang lepas. Penerokaan bakteria marin dianggap sukar dan merbahaya, tetapi hasil kajian amat berhasil dalam menyediakan maklumat bagi menjana pemahaman yang lebih baik terhadap fungsi bakteria marin di dalam air laut. Penerokaan bakteria marin di persisiran pantai Malaysia dianggap sebagai baru dan tanpa data yang berkesan untuk menerangkan taburan bakteria di perairan Malaysia, khususnya yang menghala ke Laut China Selatan. Tujuan kajian ini adalah untuk menilai kepelbagaian bakteria di perairan luar Terengganu bagi menjana maklumat awal mengenai kepelbagaian bakteria marin di persisiran pantai. Dalam kajian ini, sedimen di permukaan laut yang mengandungi sel bakteria telah diambil dari tiga lokasi rawak dengan mengambil kira kedalaman paras air yang berbeza. DNA yang diperoleh melalui proses pengekstrakan sel bakteria dikenalpasti melalui kaedah *Next Generation Sequence*, dengan mensasarkan sifat 16SrDNA V3-V4 khususnya untuk menjana keseluruhan profil metagenomik bakteria. Hasil kajian menunjukkan kepelbagaian bakteria di perairan luar Terengganu terdiri daripada 25518 amplikon daripada 3301 OTU yang unik, yang menandakan 27 filum. Kekuatan OTU semakin berkurangan dengan kedalaman sedimen di dalam laut. Profil metagenomik menunjukkan dua genus bakteria pendegradasi sulfur adalah dominan di kawasan kajian. Genus *Sulfurovum* mendominasi keseluruhan komuniti bakteria di dua lokasi yang terletak di kawasan timur laut dari stesen pensampelan. Sebaliknya, genus *Pseudoalteromonas* mendominasi komuniti bakteria di kawasan tenggara persisiran pantai. Analisis fisio-geokimia mendedahkan bahawa semua kawasan kajian mengandungi sulfur, minyak dan gris, gasolin, diesel dan minyak mineral, yang mungkin mempengaruhi pertumbuhan komuniti bakteria pendegradasi sulfur di kawasan kajian. Tidak ada bukti kukuh untuk mengaitkan *Sulfurovum* dan *Pseudoalteromonas* sebagai bakteria penyebab penyakit kepada manusia. Akan tetapi, ada kemungkinan kawasan-kawasan yang dikaji telah tercemar akibat perbuatan manusia dan analisis fisiko-geokimia lanjutan amat diperlukan. Kesimpulannya, hasil penyelidikan ini mencadangkan keperluan untuk menjalankan penyelidikan kepelbagaian bakteria yang lebih meluas, seperti skala penyebaran bakteria dan variasi komuniti bakteria untuk mengukur kadar pencemaran alam di dalam kawasan kajian.

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LIST OF ABBREVIATIONS

COD	-	Chemical Oxygen Demand
DNA	-	Deoxyribonucleic acid
DO	-	Dissolved Oxygen
DOM	-	High-Molecular Weight Dissolved Organic Matter (DOM)
HAB	-	Harmful Algal Bloom
HEM	-	Hexane Extraction Method
MEOR	-	Microbial Enhanced Oil Recovery
NGS	-	Next Generation Sequencer
NSCS	-	Northern South China Sea
NTU	-	Nephelometric Turbidity Unit
O&G	-	Oil and Grease
OTU	-	Operational Taxonomy Unit
PCR	-	Polymerase Chain Reaction
POM	-	Particulate Organic Matter (POM)
RDP	-	Ribosomal Database Project
ROS	-	Reactive Oxygen Species
TDS	-	Total Dissolved Solids
TOC	-	Total Organic Carbon
TPH	-	Total Petroleum Hydrocarbon
TSD	-	Terengganu Sediment
TSS	-	Total Suspended Solids
QC	-	Quality check
RDP	-	Ribosomal Database Project
SCS	-	South China Sea
SSCS	-	Southern South China Sea

LIST OF SYMBOLS

10^x *cells* ml^{-1}	-	(10^x) *is order of magnitude in Most Probable Number (MPN) method*
16S rDNA	-	*16 Svedberg ribosomal DNA*
bp	-	*DNA basepair*
km	-	*kilometre*
m^2	-	*square metre*
m^3	-	*cubic metre*
mg/l	-	*miligram per litre*
S	-	*Svedberg / sedimentation rate*
µm	-	*micro metre*
°	-	*degree*

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CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter describes research background, problem statements, research aims, research scopes, hypothesis, conceptual framework, and research limitations. The research background consists of short and brief information regarding the marine bacteria, information of the surveyed area, and few explanation on the necessity to conduct marine bacterial community study in the seawater, and in the proposed sampling station. Subsequently, a conceptual framework is introduced before addressing the research objectives, scopes, hypothesis, and limitation. Several critical information that requires further explanation in a different chapter are carefully mentioned (e.g. in Literature review and Methodology).

1.2 Research Background

The water interconnected body covers 70 percent of the Earth’s surface where it consists of diverse marine life. The marine ecosystem in the ocean is been existed for about 3.5 billion years, where two-thirds of its community are the marine microbes (Munn, 2011). Although microbiology diversity study in the seawater is widely studied, there is no detailed conclusion to determine marine microbial roles in the seawater because, these kinds of research are difficult construe as it involves complexity of biological affiliation issue in the seawater. Therefore, the marine microbe exploration progress brings a major hindrance to the microbiologist. For instance, cultivation of a live marine microbe outside its natural habitat is expensive and scientifically unstable. Most of the research outcomes are vacillating and it requires more cognitive approach to identify the unknown bacterium (Munn, 2011).

To date, several studies have confirmed that most of marine bacteria are a dynamic key player in the oceanic ecological system – where it regulates the biogeochemical cycle to support ecological sustainability (Hanson *et al.*, 2011; Worden *et al.*, 2015). The marine bacteria are microscopic in size and requires a selective nutrient to support their growth (Inagaki *et al.*, 2004; Takai *et al.*, 2004). There is one research has speculated that all marine bacteria consume the same nutrient compound for its energy resources (Dinsdale *et al.*, 2008). It is believed that local seawater physical-geochemical parameters may reflect a local microbial community such as: - pressure, salinity, oxygen concentration, temperature, and carbon source (Dinsdale *et al.*, 2008b). There is no concrete evidence that supports an equal marine bacteria diversity amount in a different marine environment (Munn, 2011).

Several findings show that a marine bacterium able to generate its own molecular signal, to observe its local environment. This unique and complex biological function is a useful for the marine bacterial “communication” because it regularly needs to transmit itself elsewhere: To surge its predatory skills, and permit cell modifications to protect itself in an extreme environment (Whitehead *et al.*, 2004; Gómez-Consarnau *et al.*, 2010). Investigation on local marine bacteria interaction is an ongoing process, with a purpose to improve a better deviation process; parallel to the global environmental alteration pattern (Van der Gucht *et al.*, 2007; Wang *et al.*, 2015). It is worth to mention that, a continuous research on marine microbial deviation process does illustrate a sturdier and gradual improvement: Such as, dispersion bio-geography model in various environments (Lindström & Lagender 2012; Bokulich *et al.*, 2014; Wang *et al.*, 2015).

The South China Sea (SCS): as illustrated in Figure 1.1, is a marginal sea with an average bathymetry depth of 1200m (Hogan, 2013). The SCS is considered as the golden waterway for the Eurasia with the Americas, because it provides a safe nautical route. This sea serves as a terminal for the busiest container seaports traffics in the world, where it mainly located in China, Singapore, Taiwan, and Malaysia (Fan *et al.*, 2015). The SCS shallow water contains a valuable oil and gas reserves (Ismail *et al.*, 2015), a diverse marine life (Cao *et al.*, 2007), and a rich coral reef zone (Arai, 2015).

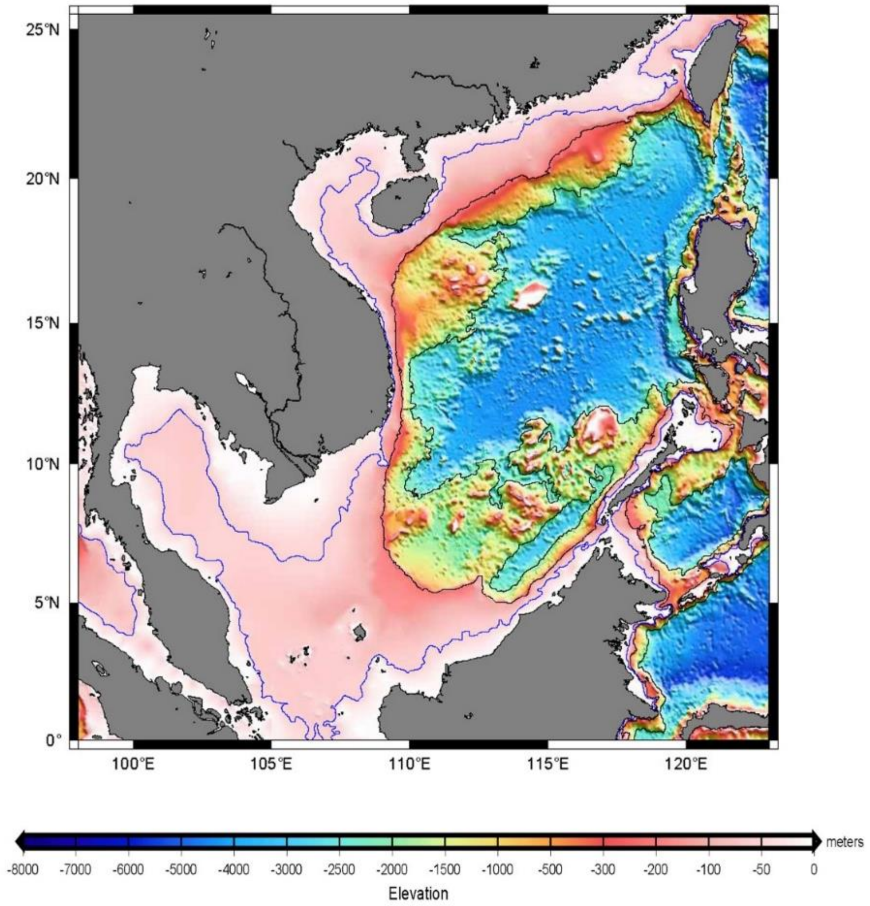

Figure 1.1 Illustration of the South China Sea bathymetry

(Image courtesy of Liu and Dittert, 2010)

Unfortunately, the SCS is notable for its dreadful cases of water pollution in several of its coastline (Rosenberg, 2009), where it is believed that mariculture activity contributes to the coastline pollution the most (Cao *et al*., 2007). For example, several coastlines in the North SCS were badly affected due to mariculture management negligence; specifically, disposing the mariculture waste. In general, mariculture waste that is discarded into the seawater will increased the COD, active phosphorous, and ammonium values; eventually, transformed a hearty coastline ecology into a “dead sea” (Feng, 1996; Cao *et al.*, 2007). A further discussion about anthropogenic pollution in the SCS can be referred in sections 2.3.2.

Prior to mariculture pollutant cases reported in the SCS coastline, the affected nations have reported several seafood poisoning cases that are mainly linked up to marine bacterial invasions such as: - Vibriosis, Pseudomonas invasion and Shewanella septic shock. Information on these diseases can be referred in sections 2.6. Before this research was conducted, numerous report that is being associated with marine bacterial infections in the affected SCS coastline was reviewed, where the result of this review is revealed in section 2.7.

However, this review was conducted with little information of physical-geochemical information available. Therefore, microbial community identification in both pristine and polluted coastlines is still difficult to predict. In this study, a comprehensive phylogenetic sequencing technology, namely Next Generation Sequencer (NGS) was utilized to describe a local bacterial community profile in three sampling points. The overcomes of this study may provide valuable information on microbial ability survivals in both normal and deprived regions.

In this study, the sampling area represents the SCS coastline, with no or minimum water intrusion occurs from the other sea region. In the Malaysian water, there are three coastlines that suitably signify the SCS coastline, which is: - Off – Terengganu coastline in Terengganu, Kota Kinabalu coastline in Sabah, and Bintulu coastline in Sarawak. The Off-Terengganu coastline are chosen as the sampling station because it is the nearest location for this study, and it is well positioned with no visible water flux influence expected to occur from the Gulf of Thailand.

The Off-Terengganu coastline is conjoined with the Kuala Terengganu river estuary, three small islands and several piers that are situated approximately two kilometres inside a curvaceous concrete breakwater. Based on a personal survey and visual information as depicted in Figure 1.2 and Figure 1.3, the Off-Terengganu coastline accommodate a moderate fishing vessel and speedboats traffics in daily basis. In addition, several water drainages are spotted in this area, where the effluent are mainly influenced by a high-density fisherman’s village, restaurant, mariculture, and hotels. Recent findings suggested that Off-Terengganu is vulnerable against anthropogenic pollutant with a notable amount of BOD, COD, TSS and, AN were reported (Suratman *et al.*, 2015; Kamaruddin *et al.*, 2016).

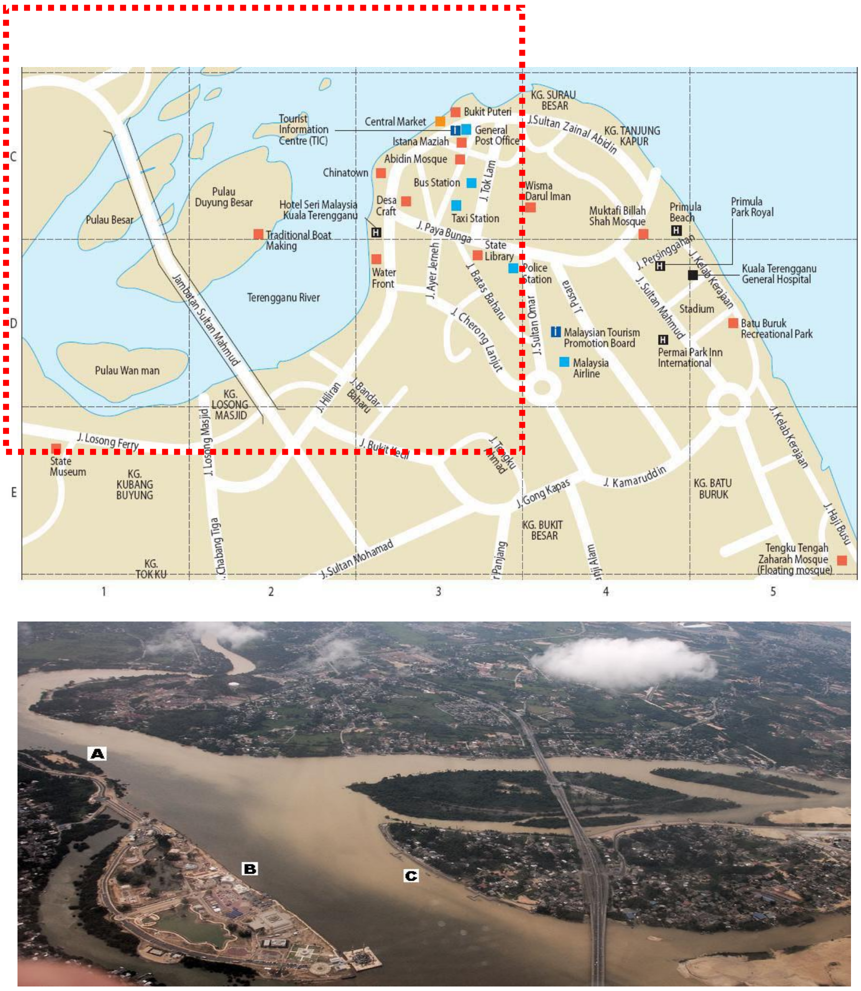

Figure 1.2 Illustration of the Pulau Duyong Besar Island (C), the Kuala Terengganu river (A), Pulau Wan Man, Pulau Besar, several hotels (B), fishing villages, and restaurants.

Given the context of possible sediment amiability towards the anthropogenic pollutant compound in the surveyed areas, the bacterial phylogeny profile in Off-Terengganu might not represent a spot-on native marine bacterial community description. Perhaps, it may illustrate a unique bacterial community that comprises several species that has its own metabolically readiness to utilize inorganic compound substrate such as: Sulfate, Carbon, and Silica. Furthermore, this research might identify a waterborne bacterium that caused infection threat to other marine community and humans (Marziah *et al.*, 2016).

Figure 1.3 Bird Eye's View of Several Piers, Drainage, and Hotels in Pulau Duyong Interconnected with Off-Terengganu Coastline

1.3 Problem Statement

Ever since marine microbe exploration was initiated fifty years ago, investigations on marine microbial diversity in the ocean, its part in ocean ecology, its interaction with other marine life and its benefits for human beings have risen greatly among microbiologists around the globe. Despite excellent pioneering on such investigation, understanding of marine bacterial diversity was somehow slow and remains indecisive (Munn, 2011).

In principal, this study aims to create a steadfast foundation about marine bacterial community in the SSCS region - specifically in Malaysia seawater. Findings that are attained from this study are critical, because it will represent the first impression of marine bacterial community in the Malaysians' water (Marziah *et al.*, 2016). A massive marine bacteria phylogenetic study was previously identified in the British channel (Gilbert *et al.*, 2012), and NSCS (Zhu *et al.*, 2013) as an effort to describe a practical bacteria community profile in its local environment. Subsequently, the outcome data have expanded global bacteria diversity coverage (Klindworth *et al.*, 2012; Gilbert *et al.*, 2012).

Identification of marine bacteria by the phylogenetic approach irrefutably reduces discrepancy in colony enumeration and taxonomy richness (Kim *et al.*, 2011; Mizrahi-Man *et al.*, 2013). Furthermore, the phylogenetic approach has revealed numerous of conspiring factors that propel a marine microbiology subject to the forefront of "mainstream" sciences; and becomes an exciting, fast-moving marine diversity research (Gilbert *et al.*, 2012; Munn, 2011).

Marine microbial ecology in the seawater requires a radical rethinking; to comprehend the oceanic eccentric, and delivers an intriguing insight of symbiosis phenomenon, food webs, and pathogenicity (Munn, 2011). Therefore, a correct methodology combination such as: phylogenetic approach, remote sensing, and sea exploration is required, in order to improve countless of data gap in the microbial diversity research. For instance, addressing the data gap in: species coverage and bacterial cell interaction in various environment condition. Currently, global marine bacteria exploration has identified approximately 44 percent of effective marine bacteria species, where it is mainly retrieved from Europe, East Asia, Middle America, Arctic Region, and the Atlantic Ocean (Gibbons *et al.*, 2013). In the South China Sea, only a minimum amount of the local marine microbial diversity data (based on phylogenetic method) is accessible. Therefore, it is hampering any efforts to compare and contribute marine bacterial diversity information in Asia with the other regions. Interestingly, the marine microbe research in the Southeast Asia region is mainly conducted in responds to seafood-related poisoning cases (Cahill, 1990; Austin, 2006; Anwar & Choi, 2014). For instance, there are several pathogenic marine bacteria have infested the fisheries products, and accidentally instigate a severe infection / mortality in the public community of Southeast Asia such as: *Bacillus* sp., *Vibrio vulfinicus*, *Shewanella* sp., and, *Pseudoalteromonas* sp. Therefore, it is essential to investigate the marine microbe's interactions in its local environment and develop an effective mitigation plan that will inhibit future outbreak (Anwar & Choi, 2014).

Bacteria cultivation is very important in the microbiology mainstream research because a bacterium cell is adjustable for a steadfast research preference and must be microscopically visible for continuous monitoring. Therefore, a pure cell culture is mainly used in the microbe susceptibility study to determine its virulence factor towards several living cells such as: - skin (Natsuga *et al.*, 2016), liver (Yeh *et al.*, 2016), brain (Wang *et al.*, 2016), blood (Moore *et al.*, 2016) etc. In addition, microbial susceptibility study helps to investigate antibiotic potential (Torres-Barceló & Hochberg, 2016) or antibiotic resistance factor (Yu *et al.*, 2016; Longo *et al.*, 2016). In recent claims, bacteria cultivation has demonstrated microbial ability to degrades dissolve or non-dissolved organic compound for energy (Thomas *et al.*, 2016; Canuel & Hardison, 2016)

The greatest challenge in marine bacteria cultivation is, by what method to imitate its growth outside its natural environment. Generally, there are notable physical-geochemical differences in the seawater, such as: - local chemical constituent, temperature, and atmospheric pressure (Alain & Querellou, 2016). Nevertheless, the success rate of obtaining a functional bacteria cell is trifling: because it is generally incapable to acclimatise in abrupt physical-geochemical changes (Suzuki *et al.*, 1997; Schut *et al.*, 1997; Cannon *et al.*, 2002)

Therefore, the microbial DNA extraction method is introduced in this study because it can be obtained from both live and dead cells. This technique reduces contaminated cell occurrences in the sample, throughout sampling, DNA extraction, and amplification (Strong *et al.*, 2014). Subsequently, the amplified DNA sequences are customarily targeted, to meet the research objectives before conducting a sequence assessment through genome depository interfaces such as: the NCBI, SILVA, and Genbank (Cole *et al.*, 2009; Pak & Kasarkis 2015). However, it is anticipated that the unknown phylum may be identified. Consequently, the unknown DNA must undergo a difficult and meticulous annealing process, before the exact sequence could be configured.

1.4 Research Objectives

i. To evaluate bacterial abundance in a selected coastline surface sedimentary layer
ii. To identify bacterial species that are dominant in a selected coastline surface sedimentary layer
iii. To identify, among those dominant species, a potential waterborne bacterium that causes disease towards the human.

1.5 Research Scope

i. This research is mainly focused on identifying a shallow benthic bacterial community from the natural coastline.
ii. Sampling is conducted in three different locations of different depths, to analyze the overall bacterial diversity in its local community
iii. The dominant genus based on the phylogenetic report is then analyzed for its interaction in the sampling area, and addressed its metabolic capability to induce infection in humans and animals.

1.6 Conceptual Framework

Implementation of the conceptual framework is essential in order to build conceptual distinction and organize research ideas effectively. Implementation of conceptual framework helps science research to advance faster and ensure every researcher to work inside an explicit framework of concepts and theories (Scheiner 2010). Historically, Suppe (1977) indicates that a conceptual framework for science always exists but never theoretically. In recent years, Scheiner (2010) believes that Suppe (1977) indication is parallel with general biological research. Generally, biology based research has no obvious predominant conceptual framework and has few general theories (Scheiner, 2010).

The conceptual framework is important because it clarifies thinking and forces a modicum of formality onto data interpretation. Scheiner and Willig (2008) believe that biologist does acknowledge only one theory - Charles Darwin's Theory of Evolutions: where these theories comprehend cells, organisms, and genetics evolution. To construct theories that represent a general biology research, it must have a potential applied it to every species with no limitation set of species. Accordingly, a fundamental principle must apply to all or most of the constitutive theories within the domain of the general theory. Those principles should work as basic assumptions behind all the constitutive theories and models, generating a link between constitutive theories. Next, the first fundamental principle of a theory should encompass the basic object of interest, and all the theory components should serve either to explain a central observation or to explore its consequences (Scheiner, 2010).

In overall, the conceptual framework for this study is constructed based on Scheiner's (2010) Towards a Conceptual Framework for Biology review, to reform formality thinking onto data interpretation, and averts any scientific disputes. Nevertheless, establishment of the conceptual framework may reveal a hidden information on specific models, or experiments where it perhaps clarifies the central questions that are being addressed by a scientific community. In this research, strategies on conceptual framework development are deliberated in the Chapter 2, section 2.7.

1.7 Limitations of Study

i. Bacterial 16S rDNA phylogenetic report only covers V3 and V4 hyper-region, which perhaps, impeding the chances to obtain targeted genus identification.
ii. Bacterial species and strain identification are not included in this study, because it requires a complex, expensive, and lengthy sequencing outline to construct a coherent cloning.
iii. Only three (3) sampling locations are selected for this study due to financial, time restriction and safety concern.

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APPENDIX A

THEORY OF BIOLOGY

Table A 1 The domain and fundamental principles of the theory of biology

Domain	
The diversity and complexity of living systems, including causes and consequences	
Principles	
1.	Life consists of open, non-equilibrial systems that are persistent
2.	The cell is the fundamental unit of life
3.	Life requires a system to store, use, and transmit information
4.	Living systems vary in their composition and structure at all levels
5.	Living systems consist of complex sets of interacting parts
6.	The complexity of living systems leads to emergent properties.
7.	The complexity of living systems creates a role for contingency
8.	The persistence of living systems requires that they are capable of change over time
9.	Living systems come from other living systems
10.	Life originated from non-life

APPENDIX B

THEORY OF CELLS

Table B1 The domain and fundamental principles of the theory of cells

Domain

Cells and the causes of their structure, function, and variation

Principles

1. Cells are highly ordered, bounded systems
2. Cells are composed of heterogeneous parts consisting of subsystems that act to localize resources and processes
3. Cells are regulated by a network of biochemical and supermolecular interactions
4. Cells interact with their external environment, including with other cells
5. Cells exchange matter through boundaries consisting of semipermeable membranes.
6. Cells require an external source, either chemical or electromagnetic.
7. Cells use energy to create concentration gradients of ions and molecules.
8. New cells are formed from other existing cells.
9. Cells contain all of the information necessary for their own construction, operation, and replication.
10. The properties of cells are the result of evolution.

APPENDIX C

THEORY OF ORGANISMS

Table C1 The domain and fundamental principles of the theory of organisms

Domain	
Individual and the causes of their structure, function, and variation	
Principles	
1.	An individual organism actively maintaines its structural and functional integrity
2.	All organism are composed of cells at some point in their life cycle.
3.	Organismal maintenance at one level requires change at other levels.
4.	Organismal functions trade-off against each other.
5.	Organismal maintenance is a functions of interactions with the abiotic and biotic environment
6.	Organisms require external sources of materials and energy for maintenance, growth, and reproductions.
7.	Because organism are changeable, external influences can force change
8.	Heterogeneity of resources in space and time leads to variation in ontogeny and life history patterns
9.	Organismal reproduction is both a cause and consequences of evolutionary processes
10.	The properties of organisms are the result of evolution

APPENDIX D

THEORY OF GENETICS

Table D1 The domain and fundamental principles of the theory of genetics

Domain	
The patterns and processes of the use, storage, and transmittal of information in organisms	
Principles	
1.	Offspring resemble their parents
2.	The fidelity of information transmittal requires an error correction system.
3.	Because life is the product of natural selection, the information system must capable to produce new information.
4.	The imperfections of error correction create new information.
5.	The exchange and recombination of information among individuals create new information.
6.	Random processes play an importance role in information transmittal, error correction, and the exchange of information among individuals.
7.	The systems of information usage must be robust to errors.
8.	Information usage is context dependent.
9.	The properties of information systems are the result of evolution

APPENDIX E

THEORY OF ECOLOGY

Table D2 The domain and fundamental principles of the theory of ecology

Domain

The spatial and temporal patterns of the distribution and abudance or organism, including causes and consequences

Principles

1.	Organism are distributed unevenly in space and time
2.	Organism interact with their abiotic and biotic environments
3.	Variation in the characteristic of organism results in heterogeneity of ecological patterns and processes.
4.	The distribution of organism and their interactions depend on contingencies
5.	Environmental conditions are heterogenous in space and time.
6.	Resource are finit and heterogenous in space and time
7.	Birth rates and death rates are a consequence of interactions with the abiotc and biotic environment.
8.	The ecological properties of species are the results of evolution.

APPENDIX F

LIST OF PUBLICATIONS

2016

Marziah, Z., Mahadzir, A., Musa, M.N., Azhim, A. and, Hara, H. (2016) Abundance of sulfur degrading bacteria in a benthic bacterial community of shallow sea sediment in the Off-Terengganu Coast of the South China Sea. *MicrobiologyOpen*. 5(X):xxx-xxx doi:10.1002/mbo3.380 (2.21)

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APPENDIX G

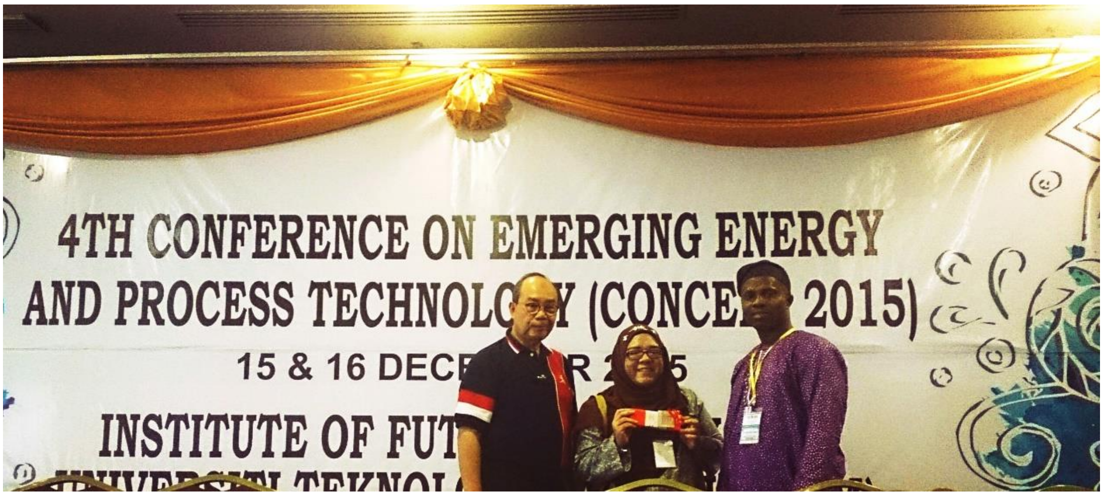
LIST OF SEVERAL BACTERIA DIVERSITY IN THE SOUTH CHINA SEA

Region	Country	Species	Host / Sample	Sampling Location
North SCS	**China**	*1.* *Aeromonas* sp. *2.* *Pseudomonas* sp. *3.* *Photobacterium* sp. *4.* *Vibrio* sp. *5.* *Enterobacter* sp. *6.* *Bacillus* sp. *7.* *Acinetobacter* sp. *8.* *Cytophaga* sp. *9.* *Lutibacteriu* sp. *10.* *Moraxella* sp. *11.* *Flavobacterium* sp. *12.* *Xanthomonas* sp. *13.* *Chromobacterium* sp. 14. *Alcaligenes* sp.	Seawater	Dapeng Bay (DP) (Jiang *et al.*, 2010)
	Philippines	*1.* *Vibrio* sp.• *2.* *Vibrio parahaemolyticus*• *3.* *Vibrio harveyi*• *4.* *Pseudomonas* sp.• 5. *Pseudomonas aeruginosa*• 6. *Pseudoalteromonas* sp.• 7. *Pseudoalteromonas viridis*• *8.* *Ruegeria lacuscaerulensis*• *9.* *Roseobacter gallaeciensis*• *10.* *Pelagibacter* sp.• *11.* *Ponticoccus* sp.• *12.* *Alphaproteobacterium*• *13.* *Halobacillus* sp.• *14.* *Bacillus pumilus*• *15.* *Microbacterium esteraromaticum*• *16.* *Algoriphagus* sp.• *17.* *Coccinimonas marina* •	Seawater•	Bolinao, Pangasinan Northern Phillipines• (Manset *et al.*, 2013)
	Taiwan	*1.* *Oceanicola marinus* *2.* *Pseudidiomarina taiwanensis* ˩ *3.* *Vibrio vulnificus* ˘ 4. *Vibrio ruber* ʷ *5.* *Vibrio fischeri*^ *6.* *Vibrio logel*^ *7.* *Vibrio harveyi*^ *8.* *Vibrio vulnificus*^ *9.* *Vibrio splendidius*^ *10.* *Vibrio orientalis*^ *11.* *Vibrio cholera*^ *12.* *Shewanella hanedai*^ *13.* *Shewanella woodyi*^ *14.* *Photobacterium leiognathi*^ 15. *Photobacterium phosphoreum*^	1. Seawater 2. Seawater ˩ 3. Seawater˘ 4. Seawaterʷ 5. Seawater^	1. Eluanbi coast, Pingtung County, (Lin *et al.*, 2007) 2. ˩An-Ping Harbour (Jean *et al.*, 2006) 3. ˘unspecified location (Goo and Wan 1995) 4. ʷKeelung (Shieh *et al.*, 2003) 5. ^unspecified location (Chiu *et al.*, 2007)

	Vietnam	1. *Bacillus* sp.+ 2. *Vibrio* sp.+ 3. *Pseudomonas* sp.+ 4. *Pseudoalteromonas* sp.+ 5. *Marinococcus* sp.+ 6. *Halobacillus* sp.+ 7. *Shewanella* sp.+ 8. *Sulfitobacter* sp.+	+Cultivated Mollusk in Seawater 1. *Crassostrea lugubris* 2. *Perna viridis*	1. + Gulf of Nha Trang Lagoon (Beleneva *et al.*, 2007)
South SCS	**West(Peninsular) Malaysia**	1. *Vibrio parahaemolyticus*♦ 2. *Bacillus megaterium* ♦ 3. *Shewanella sp.* ♦ 4. *Escherichia coli* ♦ 5. *Salinimonas chungwhensis*♦ 6. *Alteromonas sp.* ♦ 7. *Alteromonasalvinellae*♦ 8. *Pseudomonas sp.*□ 9. *Enterobacter agglomerans*□ 10. *Klebsiella pneumonia*□ 11. *Acinetobacter sp.*□ 12. *Flavobacterium* sp. □ 13. *Escherichia coli*♠ 14. *Vibrio parahaemolyticus*♠ 15. *Salmonella Typhi*♠	1. Seawater♦ 2. *Acropora cervicornis* (Coral)□ 3. Seawater♠	1. ♦(You *et al.*, 2012) 2. □East Coast of Peninsular (Kalimutho *et al.*, 2007) 3. ♠Kuantan, Pahang (Lee *et al.*, 2011)
	East Malaysia (Sabah) - West coast	1. *Vibrio harveyito*× 2. *Vibrio parahaemolyticus** 3. *Vibrio alginolyticus**	1. × Marine net cage, seawater - Asia seabass (*Latescalcarifer*) - Brown marble grouper (*Epinephelusfuscoguttatus*) - Red snapper (*Lutjanus sp.*) - Hybrid grouper (*E.fusguttatus x E. lanceolatus*) 2. * Marine net cage, seawater	1. ×Aquaculture facility, Sulaman Bay, West Coast Sabah (Albert and Ransangan 2013) 2. *West Coast Sabah (Ransangan *et al.*, 2013)
	East Malaysia (Sarawak) -West coast	1. *Faecal coliforms* 2. *Escherichia coli* 3. *Faecal coli* 4. *Faecal streptococci* 5. *Thalassospira profundimaris* (Carbazole degrader) s 6. *Kordiimonas gwangyanggensis* (closely related) *Alphaproteobacteria* OC6ST§	1. Seawater 2. Seawater s 3. Seawater §	1. Tanjung Batu beach, Bintulu Sarawak (Appan 1991) 2. Miri, Sarawak (Rani 2011) 3. § Zhulkarnain (2014)

APPENDIX H

GALLERY: BEST ORAL PRESENTER (CATALYST II) - CONCEPT 2015



APPENDIX I

GALLERY: SAMPLING ACTIVITY IN OFF-TERENGGANU