BACTERIAL DIVERSITY AS AN INDICATOR OF SOIL PERTURBATION AND SOIL AGGREGATE STABILITY IN THE KELANTAN RIVER BASIN

RENNIELYN RUPERT

THESIS SUBMITTED IN FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE

**BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH**

2020

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL : **BACTERIAL DIVERSITY AS AN INDICATOR OF SOIL PERTURBATION AND SOIL AGGREGATE STABILITY IN THE KELANTAN RIVER BASIN**

IJAZAH : **SARJANA SAINS**

BIDANG : **BIOTEKNOLOGI**

Saya **RENNIELYN RUPERT**, sesi pengajian **2018-2020**, mengaku membenarkan tesis Sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

[ ] SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)

[ ] TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

[/] TIDAK TERHAD

Disahkan oleh,

ALBERTO ANING
PUSTAKAWAN KANAN
UNIVERSITI MALAYSIA SABAH

RENNIELYN RUPERT
MZ1721014T

(Tandatangan Pustakawan)

Tarikh : 12 Jun 2020

(Prof. Madya Dr. Kenneth Francis Rodrigues)
Pengerusi JawatanKuasa Penyeliaan

DECLARATION

I hereby declare that the material in this thesis is of my own effort, except for quotations, excerpts, equations, references and summaries, which have been duly acknowledged and cited clearly its sources.

20 March 2020

Rennielyn Rupert
MZ1721014T

CERTIFICATION

NAME : **RENNIELYN RUPERT**

MATRIC NO : **MZ1721014T**

TITLE : **BACTERIAL DIVERSITY AS AN INDICATOR OF SOIL PERTURBATION AND SOIL AGGREGATE STABILITY IN THE KELANTAN RIVER BASIN**

DEGREE : **MASTER OF SCIENCE**

FIELD : **BIOTECHNOLOGY**

VIVA DATE : **20 MARCH 2020**

CERTIFIED BY;

Signature

SUPERVISORY COMMITTEE

1. **CHAIRMAN OF SUPERVISORY**
   Assoc. Prof. Dr. Kenneth Francis Rodrigues

2. **COMMITTEE MEMBER**
   Mdm. Grace Joy Chin Wei Lie

3. **COMMITTEE MEMBER**
   Dr. Kogila Vani Annammala

ACKNOWLEDGEMENT

Praise and thanks to The Almighty God for showering me with strength and blessing throughout my journey in completing my master degree. I would like to express my deepest gratitude to my supervisor, Assoc. Prof. Dr. Kenneth Francis Rodrigues, who has provided me with insight and expertise towards my research and assisted me from the beginning until the end of the project. Your support, comments and guidance have greatly improved my laboratory skills and writing.

I would also like to express a sincere thanks to my co-supervisors, Mdm. Grace Joy and Assoc. Prof. Dr. Kogila Vani (UTM's senior lecturer), who have been providing me guidance and knowledge in my laboratory works and writing. Your assistance and kindness have tremendously helped me throughout my studies.

I am also immensely grateful to all BRI lecturers who have shared their precious knowledge and experience to the student, especially during the session of post-graduates student seminar. Fellow staffs who are working in the administration and laboratory sections, thank you for the guidance. Also, to my laboratory mates and friends for their endless motivation, encouragement and advice to me whenever I was facing hardships.

Not to forget my family that has always been supported me financially and mentally. Lastly, I would like to thank myself for being perseverance all the time. This journey has transformed me into a brand new person.

Rennielyn Rupert
20 March 2020

ABSTRACT

The soil perturbation in Kelantan, Malaysia, is mainly caused by the logging activity and land conversion over the years. The perturbation has led to a change in soil dynamics and stability, which is one of the factors that contribute to flooding. This study utilized bacterial diversity as an indicator to investigate the soil changes related to soil dispersion in the Kelantan river basin (KRB). The bacterial diversity pattern at the four selected study points (upstream to downstream, river basin) particularly in primary forest, logged forest, oil palm plantation and rubber plantation was characterized via two molecular approaches, the 16S rDNA culture-dependent and culture-independent 16S rRNA-based metagenomic analysis. The results showed the phyla of Actinobacteria, Acidobacteria, Proteobacteria, Chloroflexi, Crenarchaeota and Verrucomicrobia dominated in the four sampling sites. Through the studies, the data revealed three locations shared similar soil bacterial composition profiles such as the phyla of Actinobacteria, Acidobacteria and Proteobacteria. The findings suggested there were an interchange of soil between diverse areas and implied that floodwaters played a significant role in perturbation of soil in the river basin. Another crucial finding was the discovery of marine bacteria in the logged forest which was previously notified as flooding areas suggesting there was an interchanged of bacteria between the marine and terrestrial land. Besides, the present study found several biofilm-producing bacteria isolated from the KRB area, which were reported in previous studies to involve in soil aggregation and stability. The characterization of biofilm-producing bacteria has provided a profound understanding of the biofilm formation process and phenotypic differences between the planktonic and biofilm forms. This study deduced that bacterial diversity could be utilized as an indicator in providing forensic, environmental evidence regarding soil dispersion and essential for land management and strategy development in the river basin, Kelantan. Also, this indicator can be extended to other regions of the world with similar patterns of anthropogenic impact.

Keywords: soil perturbation, soil dispersion, Kelantan river basin, bacterial diversity, biofilm

ABSTRAK

KEPELBAGAIAN BAKTERIA SEBAGAI PETUNJUK KEPADA PERTURBASI TANAH DAN KESTABILAN AGGREGASI TANAH DI LEMBAH SUNGAI KELANTAN

Perturbasi tanah di Kelantan, Malaysia disebabkan oleh kegiatan penebangan dan penukaran tanah telah dilakukan sejak bertahun-tahun. Perturbasi telah membawa kepada perubahan dalam dinamik dan kestabilan tanah yang merupakan salah satu faktor yang menyumbang kepada fenomena banjir. Kajian ini menggunakan kepelbagaian bakteria sebagai petunjuk untuk menyiasat perubahan yang berkaitan dengan penyebaran tanah di lembah sungai Kelantan (KRB). Corak kepelbagaian bakteria di empat lokasi terpilih (hulu ke hilir, lembangan sungai) terutamanya di hutan primer, kawasan pembalakan, ladang kelapa sawit dan ladang getah dicirikan melalui dua pendekatan molekular, analisis 16S rDNA bersandar-kultur dan metagenom berasaskan 16S rRNA tak bersandar kultur. Hasilnya menunjukkan kepelbagaian dalam empat tapak pensampelan didominasi oleh filum Actinobacteria, Acidobacteria, Proteobacteria, Chloroflexi, Crenarchaeota *dan* Verrucomicrobia. *Melalui analisis, data mendedahkan terdapat tiga lokasi yang berkongsi profil komposisi bakteria tanah yang serupa contohnya filum* Actinobacteria, Acidobacteria *dan* Proteobacteri. *Dapatan kajian memaparkan terdapat aktiviti pertukaran tanah di antara lokasi yang berbeza dan ini menunjukkan bahawa banjir memainkan peranan penting dalam perturbasi tanah di lembah sungai. Satu lagi penemuan penting ialah penemuan bakteria marin di kawasan pembalakan yang telah diklasifikasikan sebagai kawasan banjir. Kawasan ini menunjukkan ada pertukaran bakteria di antara tanah marin dan daratan. Kajian ini juga mendapati beberapa bakteria yang telah diisolasi daripada kawasan KRB mempunyai kebolehan untuk menghasilkan biofilem. Bakteria pembentuk biofilem ini dilaporkan terlibat dalam meningkatkan pengagregatan dan kestabilan tanah. Pencirian bakteria penghasil biofilem telah memberikan lebih banyak pemahaman dalam proses pembentukan biofilem dan perbezaan fenotip antara bakteria bentuk planktonik dan biofilem. Kajian ini mencadangkan bahawa kepelbagaian bakteria boleh digunakan sebagai penunjuk dalam menyediakan bukti forensik kepada alam sekitar mengenai aktiviti penyebaran tanah. Teknik ini juga penting untuk pembangunan strategi pengurusan tanah di lembangan sungai, Kelantan. Ini juga boleh diperluaskan ke kawasan lain di dunia yang mempunyai corak kesan antropogenik yang serupa.*

Kata kunci: perturbasi tanah, penyebaran tanah, lembah sungai Kelantan, kepelbagaian bakteria, biofilem

3.2.3 Differential Media Test 24
3.2.4 Genomic DNA Extraction 25
3.2.5 Amplification of 16S rRNA Gene 26
3.2.6 Sequencing Analysis 27
3.3 Culture-Independent Method 27
3.3.1 DNA Extraction from Soil Samples 27
3.3.2 Library Preparation and Sequencing 29
3.3.3 Data Analysis 30
3.4 Screening for Biofilm-producing Bacteria 33
3.4.1 Preparation of Initial Bacterial Suspension 33
3.4.2 Quantification of Biofilm via Crystal Violet Assay 33
3.5 Morphology Observation using a Scanning Electron Microscope (SEM) 34
3.5.1 Preparation Sample for Scanning Electron Microscope Observation (Planktonic and Biofilm Cells) 34
3.5.2 Scanning Electron Microscope Observation 34

CHAPTER 4: RESULT AND DISCUSSION 35
4.1 Soil Bacterial Culturing 35
4.2 Isolation and Characterization of Cultivable Soil Bacterial Assessed by Culture-Dependent Method (CDM) 36
4.2.1 Serial Dilution of Soil Samples for Bacterial Isolation 36
4.2.2 Plate Count of Colonies Formation (CFU/mL) 37
4.2.3 Differential Media Test of Soil Isolates via Plate Assay 39
4.3 Identification of Cultivable Soil Bacteria using 16S rRNA Gene Sequencing 41
4.3.1 Isolation of Genomic DNA 41
4.3.2 PCR Amplification and Sequencing on the Basis of 16S rRNA Gene 42
4.3.3 Sequencing Analysis of BLAST Output Data 44
4.3.4 Clustering Analysis (Phylogenetic Tree) 48
4.4 Bacterial Diversity (Richness and Abundance) Assessment Based on Metagenomic Approach 53
4.4.1 Sequencing and Data Depth Assessment 54

4.4.2 Bacterial Community Structure Assessment in Four Sampling Sites 58
4.4.3 Comparative Diversity between Sample Points through Metagenomic Analysis 63
4.4.4 Data Correlation of Beta Analyses 67
4.5 Biofilm-forming Bacteria in Kelantan River Basin Link to Soil Aggregate 69
4.5.1 Biofilm Formation via Crystal Violet Assay 70
4.5.2 Soil Bacterial Classification 74
4.5.3 Biofilm Observation on scanning electron microscope 76

CHAPTER 5: CONCLUSION 81

REFERENCES 83

APPENDICES 110

LIST OF TABLES

			Page
Table 3.1	:	Details and Data Coordination of Sampling Sites in the River Basin, Kelantan.	22
Table 3.2	:	List of Indices used for Species Diversity Analysis in Culture-Independent Methods.	32
Table 4.1	:	Plate Count of Soil Sample Collected from KRB.	37
Table 4.2	:	Total CFU (CFU/ml) Counts in Each Sampling Sites after 6 Days Incubation.	38
Table 4.3	:	Preliminary Identification via Four Different Types of Media	40
Table 4.4	:	The Closest Species of Subject Sequences compared to the Query Sequence in the GenBank, NCBI.	47
Table 4.5	:	The Indices of Alpha Diversity at the 97 % of Clustering Threshold.	56
Table 4.6	:	Biofilm Assay Images along with the Optical Density of Selected Strains Capable in Forming In-Vitro Biofilm on Polyethene Tubes.	72
Table 4.7	:	List of Isolates and Their Respective Pathogenicity Classification and Biofilm Formation Capacity.	75
Table 4.8	:	The SEM Images of Planktonic and Biofilm Cells	78

LIST OF FIGURES

			Page
Figure 3.1	:	Four Main Points of Sampling Sites in the Kelantan River Basin. From top: PF (Primary Forest/ High Conservation Value Forest), LF (Logged Forest), RP (Rubber Plantation), MOP (Mature Oil Palm Plantation) and NOP (New Oil Palm Plantation).	21
Figure 4.1	:	Soil Bacterial Culturing in 30 ml of Nutrient Broth (NB) by Placing in the Cell Culture Incubator Shaker for Rapid Bacterial Growth.	36
Figure 4.2	:	Genomic DNA of Isolates from Sample HCV. The bands are showing a consistent band's size approximately 1kb. A 1kb DNA marker (Promega, USA) was used to measure the band's size.	41
Figure 4.3	:	PCR Products of Isolates from HCV Sample. The bands are showing a consistent band size of approximately 464 bp. The DNA marker used is a 100bp DNA ladder (Promega, USA).	43
Figure 4.4	:	Clustering Analysis via the Formation of the Phylogenetic Tree of Bacterial Isolates from Sampling Sites at HCV, LF, RP and OP.	49
Figure 4.5	:	Rarefaction Curves. Each curve represented a sample and coloured and shaped by each sample name as labelled in the figure. The X-axis is representing the sequences number, whereas the Y-axis is representing the observed OTUs number. (HCV=High conservation value forest/ primary forest, LF= Logged forest, OP= Oil palm plantation, and RP= Rubber plantation)	57
Figure 4.6	:	Species Relative Abundance in Phylum Level. The X-axis plotted the bar chart as the "Samples Name" and Y-axis as the "Relative Abundance". "Others" with yellow colour representing a total relative abundance of the rest phylum except the top 10 phyla. (HCV=High conservation value forest/ primary forest, LF= Logged forest, OP= Oil palm plantation, and RP= Rubber plantation)	59
Figure 4.7	:	Principal Component Analysis (PCA). Each point represented a sample which was plotted by the Y-axis as the second principal component and the X-axis as the first principal component. The group coloured each sample. (HCV=High conservation	64

value forest/ primary forest, LF= Logged forest, OP= Oil palm plantation, and RP= Rubber plantation)

Figure 4.8 : Non-metric Multi-Dimensional Scaling (NMDS). The graph shows that each data point stands for a sample. Samples belong to the same group are in the same colour. The distance between data points is reflecting the extent of variation of each sample. (HCV=High conservation value forest/ primary forest, LF= Logged forest, OP= Oil palm plantation, and RP= Rubber plantation) 65

Figure 4.9 : Unweighted Pair-group Method with Arithmetic Mean (UPGMA) Cluster Tree Based on Unweighted Unifrac Distance. The tree plotted with UPGMA tree on the left side and the relative abundance in phylum map on the right side. 66

LIST OF ABBREVIATIONS

rDNA	-	Ribosomal Deoxyribonucleic acid
rRNA	-	Ribosomal Ribonucleic acid
EPS	-	Exopolysaccharide
KRB	-	Kelantan River Basin
exDNA	-	External DNA
CIMs	-	Culture-Independent Methods
CDMs	-	Culture-Dependent Methods
CFU	-	Colony-forming Unit
NGS	-	Next Generation Sequencing
HCV	-	High Conservation Value Forest
LF	-	Logged Forest
RP	-	Rubber Plantation
OP	-	Oilpalm Plantation
NA	-	Nutrient Agar
NB	-	Nutrient Broth
gDNA	-	Genomics DNA
OTUs	-	Operational Taxonomic Units
QIIME	-	Quantitative Insights Into Microbial Ecology
FLASH	-	Fast Length Adjustment of Short Reads
MUSCLE	-	Multiple Sequence Comparison by Log- Expectation
BLAST	-	Basic Local Alignment Search Tool
RDP	-	Ribosomal Database Project
PCA	-	Principal component analysis
NMDS	-	Non-metric Multidimensional Scaling
UPGMA	-	Unweighted Pair-group method with Arithmetic Mean
OD	-	Optical Density
SEM	-	Scanning Electron Microscope

LIST OF FORMULA

			Page
Formula 3.1	:	$\text{CFU/ml} = \frac{\text{(Average count)}}{\text{(Dilution Plated)(mL plated)}}$	23

LIST OF APPENDICES

			Page
Appendix A	:	Agarose Gel Images of DNA Extractions for Culture-Dependent Method.	110
Appendix B	:	Agarose Gel Image of PCR Products (16S rDNA Culture-Dependent).	112
Appendix C	:	DNA Extraction Products (Culture-Independent 16S rRNA-Based Sequencing).	114
Appendix D	:	Optical Density Values of the 48 Isolates Measured by Spectrophotometer.	116

CHAPTER 1

INTRODUCTION

1.1 Research Background

Soil is one of the most valuable resources that constitutes of solid, liquid and gaseous phases. It may consider as a renewable natural resource that has a prolonged rate of formation (Fitzpatrick, 1986). It covers only 6% of the Earth's land surface yet provides astronomical purposes to the earth including maintaining the earth's ecosystem and climates, filtering water and providing shelter to billions of living organisms such as animal, plant and microorganism that are regarded as life support-functions (Soil Science Society of America, 2013). The ability of how well the soil to perform these functions depends upon the state of the soil conditions. Previously by Kime (2012), he described a good and high soil quality as a fertile soil with excellent physical characteristics and biologically active.

Nonetheless, to preserve a good quality soil is rather challenging as the on-going global changes have always endangered it. A study by Wilkinson *et al.* (2018) reported that the intensification and expansion of land use such as urbanization and agricultural activities involving the conversion of natural forest into plantation monocultures have always been the top list in influencing the soil quality negatively particularly in soil physical structure. These activities give an impact to the land, which has resulted in the increasing rates of soil perturbation (Nang, 2015). These conditions, in turn, lead to other serious consequences such as loss of soil productivity and fertility, recurrent drought, reduction of soil microorganism species and further soil quality depletion (Caravaca *et al.*, 2002; Delelegn *et al.* 2017).

For instance, the most frequent occurrence of flooding due to climate changes and anthropogenic activities are at the Kelantan river basin (KRB) areas. Over the years, the soil quality in Kelantan has degraded drastically, and this has become a pivotal factor to the cause of a massive flood in Kelantan, 2014 (Ismail and Haghroosta, 2018). On the 18th until 24th December 2014, Malaysia, particularly in Kelantan, had received hefty rainfall. During the period, the amounts of rainfall recorded were 1726mm at Stesen Gua Musang, 1045mm at Stesen Kuala Krai, and 405mm at Kota Bharu (Tahir, 2015). Generally, two streams in KRB overflowed into a river and caused the water to flood the proximity lands (Yahaya *et al.*, 2015). The rainfall alone did not drive the flooding, but the soil conditions in the areas were responsible for the incident too. Land-clearing activities are vigorously conducted along the river basin (Asmat *et al.*, 2015). These have indirectly affected the soil structure and soil physical properties in terms of water retention capacity, compaction and aggregate stability, which have caused the soils to be more susceptible to soil erosion and flooding (Haghighi *et al.*, 2010).

The persistent threat of soil perturbation driven by climatic and anthropogenic forces has prioritized the development of management practice for soil protection, especially in urban areas. In this respect, the implementation of a reliable biological-related indicator was emphasized to monitor and provide an early warning of potential soil perturbation in an area. According to Kime (2012), an indicator should be from an easy measurable tool that enables the monitoring of the soil conditions. Besides, a technique to improve soil physical structure impacted by anthropogenic activities should be focused too. This study proposed the application of bacterial diversity as an indicator to detect the possibilities of soil perturbation incidents in different forests and land use areas. As well as to screen and characterize biofilm-producing bacteria which can enhance the aggregation and stability of deteriorated soils as reported in previous studies.

Soil microorganisms are actively involved in the soil quality through their activities within the soils. They maintain soil quality by improving the water holding capacity, rooting and growth, carbon storage, essential nutrients cycling, pollutant filtration and conservation of species biodiversity in the soil (Keesstra *et al.*, 2012; Nannipieri *et al.*, 2017). However, the alteration of soil including the changes in physical, chemical and biological properties due to humankind's activities may easily disrupt the communities within the soil (Drenovsky *et al.*, 2010; Jangid *et al.*, 2011). The alterations have influenced the composition and distribution of the soil microbial communities, which made them as one of the critical and sensitive indicators to assess soil perturbation. In the previous study by García-Orenes *et al.* (2016), the authors reported the soil microorganisms was proven as a rapid indicator of soil changes mainly in the detection of changes in soil structure and species diversity.

In order to enhance the physical properties of a damaged soil, such as its structure, porosity, aeration and water infiltration, soil bacteria are introduced and grown to the damaged land to form soil aggregations (Zhong and Cai, 2007). The secondary particles formed through the combination of mineral particles with organic and inorganic material are known as the aggregates. For example, the soil microbial such as bacteria and fungi helps in the formation of water-stable soil aggregates. They secrete sticky exopolysaccharides that glue soil particles into an aggregate, which helps in retaining soil particles together. High aggregate stability soils withstand better disruptive force such as water compared to weak aggregate soils that disintegrate quickly under erosive forces (Soil Science Society of America, 2019).

This study aimed to evaluate the soil changes associated with soil dispersion that might appear in several proximity locations of KRB by utilized bacterial diversity served as a biological indicator. The metagenomics 16S rRNA sequencing approach was applied to identify the bacterial diversity from each of the sampling points. Apart from that, this study also aimed to screen and characterize bacterial species associated with exopolysaccharides (biofilm) production that secretes cementing agents via culture-dependent method along with crystal violet biofilm assay (quantitative) and scanning electron microscope (qualitative). The scope of the study for the investigation of soil perturbation in the KRB was only limited to four sampling sites, which are the primary forest, logged forest, rubber plantation and oil-palm plantation. For the screening of biofilm-forming bacteria, it was only limited to the testing of *in vitro* biofilms on the abiotic surface (polyethene surface).

1.2 Research Hypothesis

Hypothesis 1: The bacterial communities associated with soil can be utilized as an indicator in environmental forensics.

Hypothesis 2: Soil bacteria can produce biological films consisting of exopolysaccharides (EPS) and biofilm.

1.3 Research Objectives

The objectives of this study are to address the research hypotheses, as stated above.

The objectives are:

1. To identify the bacterial diversity in soil based on culture-dependent 16S rRNA gene sequencing,
2. To determine the bacterial diversity in soil using culture-independent 16S rRNA metagenomics sequencing and,
3. To characterize the bacteria associated with exopolysaccharide production by 16S rRNA sequencing and scanning electron microscope (SEM).

CHAPTER 2

LITERATURE REVIEWS

2.1 Malaysia Soil Profiles Reference to Kelantan

The literature review has focused on the soil microbial metagenome and the manner in which it can be applied as a tool for soil forensics. As well as to review on the importance of soil bacteria with biofilm-producing ability, that can be translated into soil particles' adhesive agent for aggregates stabilization. The present study used the Kelantan river basin (KRB) as a model site to test the research hypotheses. Studies on the soil profile in Malaysia have been carried out since the last decades. McWalter initiated this study in 1956, in which the work was concentrated primarily on the soil at the plantation land in the year of 1955. Between 1950-1960 and 1968-1969, he conducted the soil surveys in Kelantan and recorded the data regarding the morphological and chemical properties of the soils. The data revealed the soil in Kelantan as a complex land surface that is composed of unconsolidated quaternary marine and fluviatile alluvium underlined by the bedrock of granite and sedimentary rock (Hoong and Kiat, 1975). Morphologically, the soil in Kelantan has a dense texture and formed excessive drainage soil that created the formation of back swamps along the river (Paramananthan, 1989). These back swamps were developed due to the soil erosion that occurred in close proximity to a river.

The socio-chemical properties of soil in Kelantan were summarised according to the data reported by Paramanathan (1989). The data presented the clay content deposited at the marine, river estuarine and brackish exceed 50% and the percentage gradually decreased in riverine soils. Soil near the marine has a pH of 4.0-5.0 whereas soil in brackish water reported to actively producing acid sulfate soil resulting in a lower pH, ranging around 3.0-4.0. The data also reported on the status of soil fertility in Kelantan. Generally, the productivity of the soil in an area is determined based on the cation exchange capacity, which is also highly dependent on the mineralogy of the grounds. The soil in Kelantan displays low exchangeable cations signified a lower fertility status in the area.

In Kelantan, the river basin is among the largest basin in Malaysia, which is occupied more than 80% of the state and located in northeastern peninsular Malaysia. At the upstream regions (e.g. Gua Musang, Jeli and Kuala Krai), the basin is mainly dominated by 60 % of conserved tropical rainforest, 12 % of rubber estates and 10 % of oil palm plantations. Whereas at the downstream regions (e.g. Kota Bahru, Pasir Mas, Tumpat and Machang), the basin is occupied by 1 % of paddy field, 1 % of other agricultures and 1 % of town areas (Faizalhakim *et al.*, 2017). The soil type at the mountain ranges in the eastern and western regions of the basin is mostly composed of granite soils whereas the northern part is mainly peat and silt soils (Tan *et al.*, 2017). In previous studies conducted by Bamaiyi *et al.* (2016) and Wong *et al.* (2018), the authors reported on the frequent occurrence of monsoon flood in Kelantan. These massive floods are usually occured during the northeast monsoon season (November to January), in which Kelantan has been reported as the most affected state.

A rainfall trend analysis by Adnan and Atkinson (2011) stated the basin shows a dramatic rainfall amount during the wet season and decreases on the dry season. The increase of rainfall during the monsoon season has caused the imbalance of the input and output of water discharges, which has resulted in a massive volume of water to overflow from the riverbank and flood the floodplains in Kelantan. A similar observation by Yahaya *et al.* (2015) also reported on the occurrence of flooding in the KRB regions. It stated that due to the geographical location of the river basin that situated at the adjacent of the coast of the South China Sea and the development of settlements at the plain topography of the river basin, it has raised the exposure of the land in Kelantan to flood, especially during the northeast monsoon season from November to March.

The existence of the natural disaster is not solely induced by the heavy precipitation alone, but the soil structure in the river basin need to be considered too. Activities such as deforestation, agricultural land expansion, urbanization and industrialization can alter the soil-landscape leading to the phenomenon of flood in the Kelantan (Tan *et al.*, 2017). For instance, the conversion of natural forest into agricultural lands may reduce soil aggregations, as agricultural plants do not have the strength to hold onto soil particles. As a result, this can affect the soil physical structure at the converted regions. Moreover, the topsoil of the land may experience drought due to the less coverage and high exposure of sun which diminishes the soil microorganisms that are responsible for keeping the soil fertile (Stirton, 2019).

2.2 The Roles and Importance of Soil Microbes

Documentary evidence pertaining to the importance of soil microorganisms has been presented in many studies. In one gram of fertile soil may comprise billions of living organisms, including soil microorganism. These soil microbes contain bacteria as the most effective and abundant microorganism, actinomycetes, fungi, soil algae, soil protozoa and viruses in numerical order. These groups of microbes have distinct characteristics and function differently whereby they live and survive as a community in the soil, and some form a synergistic interaction for a living (Gupta, 2011). They ensure the biogeochemical cycles in the earth to function effectively, which fundamentally crucial to the life and sustainability of the earth.

In the biological context, bacteria are microscopic organisms that are ubiquitous components of soils. In every gram of fertile soil, it contains about 100 million to 1 billion bacterial cells estimated at around 60,000 different species, and each performs uniquely according to their functions and capabilities in soil. Some of the species are vulnerable by means they are easily destroyed by slight environmental changes such as extreme drying, cold and heat. While some portray a distinctive feature, where they can survive under severe environmental conditions, and some can experience a dormant mode for decades while waiting for their favourable circumstances. These bacterial cells can be categorized into several groups depending on their roles in soil. For instance, they can be classified as a decomposer, nitrogen fixer, disease suppressor, aerobe and anaerobe, actinobacteria and sulfur oxidizer (Reid and Wong, 2005).

Bacteria grow under different environments and specific niche in the soils. They perform countless notable roles and ecosystem services, including intensifying soil structure, recycling nutrients and recycling water in the soil. They enhance the soil conditions to support new plants to grow and live. For instance, the plant growth-promoting rhizobacteria (PGPR) promotes plant growth by associating with the biological activities in the rhizosphere areas. A rhizosphere may refer to the regions surrounding the root system of a plant (Zeppenfeld *et al.*, 2017). These PGPR bacteria promote plant growth by secreting growth regulators (hormones), increasing essential nutrients while strengthening the plant's defence system against plant pathogens (Kumar *et al.*, 2016). The rhizobacteria genera, such as *Azospirillum*, *Pseudomonas* and *Bacillus*, are all broadly explored commercial rhizosphere colonizing bacteria (Bhattacharyya and Jha, 2012; Karthik *et al.*, 2016). Without the presence of these soil species, plants are difficult to survive in complex climate conditions. They support the growth of plants by modifying the environment of the soils into a more favourable condition for the plants to flourish.

Another example of plant-promoting bacteria is the photosynthetic bacteria that colonize in the soil and performs nutrient cycling such as nitrogen, carbon, and phosphorus cycles to produce organic matter, which is beneficial for the growth of plants (Ingham, 2009). Nitrogen is an essential macronutrient for plant growth and development. Nevertheless, nitrogen is not abundantly available in the soil due to the regular nitrogen loss. Hence, these bacteria play a crucial task in the nitrogen cycle by changing the nutrient from the rare form into a usable form and supplement it to the plants (Johns, 2017; Mhatre *et al.*, 2019). Apart from that, phosphorus is another crucial nutrient for plant growth. This nutrient only exists in an insolubilize form. Hence, it requires the photosynthetic bacteria to convert it into a solubilized form via the secretion of a specific enzyme, chelation, production of organic acid and acidification (Gulati *et al.*, 2010). Through the roles mentioned above, soil bacteria have shown their importance in providing essential nutrients in the soil.

Aside from bacteria, actinomycetes are also involved in numerous biological activities and processes in the soil. Depending on the environmental state, about 10 to 30 % of soil microorganisms in the rhizosphere are comprised of Actinobacteria and actinomycetes (Sylvia *et al.*, 2005). Actinomycete has a unique feature in which it is recognized as a Gram-positive bacteria with a high content of guanine and cytosine (>50). Still, it has an almost similar morphology to fungi. It forms branched filamentous hyphae that resemble the character of fungi and lives actively in a high soil pH environment (Ingham, 2009; Nayaka *et al.*, 2017). Interestingly, this microbe is responsible for creating the earthy smell from the soil. For instance, the genus *Streptomyces* produce a unique, organic compound known as the geosmin after the death of the soil actinobacteria. The production of the geosmin has generated the smell of the earthy aroma. Besides that, they also involved in other roles such as the production of humus to improve soil structure, secretion of essential nutrients and enhancement of water retention in soil structure (Hoorman, 2016).

While microbiologists are paying extra attention to the study of bacteria, fungi have been revealed to have equivalent value as bacteria. Fungi are eukaryotic organisms that encompass a diverse group of organisms, including single-cell microorganism such as yeast to macroscopic multicellular organisms, such as mushroom species (Schoch *et al.*, 2014). Fungi may consider as one of the most abundance eukaryotes in the earth (Hawksworth, 2015). They are essential to the ecosystem, particularly for nutrients cycling and breaking down plant materials that are involved in many enzyme systems. Due to this ability, fungi are recognized as one of the primary decomposers in the forests. Other than that, fungi can produce numerous beneficial secondary metabolite products that are widely utilized in the biotechnology industry (Bandara *et al.*, 2015). For instance, some of the secondary metabolites can exhibit beneficial effects to the plants particularly at promoting the growth of roots and shoots, improving the resilience towards environmental stresses and inducing systemic resistance (ISR) of plants from any potential infection pathogens (O'Brien, 2017).

There are three main functions of fungi, which are as a decomposer, mutualist and parasite. Soil fungi are beneficial, but they can also bring harm to