A STUDY OF SOIL BACTERIAL COMMUNITIES OF FILDES PENINSULA, KING GEORGE ISLAND (SOUTH SHETLAND ISLANDS), ANTARCTICA

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ABSTRACT

STUDIES OF THE SOIL BACTERIAL COMMUNITY IN THE ANTARCTIC PENINSULA USING POLYMERASE CHAIN REACTION-DENATURING GRADIENT GEL ELECTROPHORESIS (PCR-DGGE)

There is sparse information on the bacterial diversity of Filldes Peninsula, King George Island of the maritime Antarctic. A metagenomic approach was used in this study to determine the dominant bacterial population in the soil from the lakes, river and glacier at the Fildes Peninsula, King George Island. Total of ten soil and sediment samples were studied for bacterial diversity using the PCR-DGGE approach targeting for the 16S rRNA gene. About 0.12 to 4.80 µg of DNA was extracted from one gram of soil using the hot enzymatic direct lysis DNA extraction method. Partial 16S rRNA gene product was amplified using the GC357f and 907r primer set, and the amplicon was further resolved using DGGE. Ten to 100- fold dilution of the template or an addition of 10 µg/µl of bovine serum albumin (BSA) in the PCR reaction had successfully reduced the inhibitory effect of the contaminants on the *Taq* polymerase. All the soil samples have different profiles of predominant bands. The predominant bands were excised for sequence determination and bacterial identification. A total of 99 bands and 299 clones were selected from ten locations: Antarctic Lake (AL), GFZ Lake (ZL), Estrellas Lake (EL), Playa Elefantes (PE), Minas River (MR), Collins Glacier (CG), Kitiesh Lake (KL), Belem Lake (BL), Geografos Lake (GL) and oil tank area (OT). After grouping these sequences in different operational taxonomic units (OTUs) (similarity <97%), they were categorized into RDP-designated phylum: Bacteroidetes (27.4%), Proteobacteria (25.7%), Acidobacteria (13.1%), Gemmatimonadetes (4.0%), Firmicutes (4.0%), Actinobacteria (3.4%), Chloroflexi (1.7%), Nitrospira (1.1%), Cyanobacteria (1.1%), WS3 (1.1%), Deionococcus-Thermus (0.6%), Spirochaetes (0.6%) and BRC1 (0.6%). Another 15.4% of the sequences were grouped into unclassified bacteria. Almost 90% of the OTUs have closest relative with uncultured bacterium from the NCBI GenBank database. About 79% of the OTUs had been retrieved in regions which were outside from the Antarctic continent. Acidic soils demonstrated lower diversity of bacteria where two weakly acidic soils PE and OT had the lowest Shannon diversity index. There was no obvious correlation for the changes of bacterial communities between those areas influenced by human activities and less-disturbed by human activities except in the oil tank area. Several phylotypes affiliated with hydrocarbon-degrading bacteria were detected in oil tank area.

ABSTRAK

Maklumat tentang keanekaragaman bakteria jarang ditemui di semenanjung Fildes, Pulau King George, kepulauan Antartik. Oleh itu, pendekatan metagenomic telah digunakan untuk menentukan populasi bacteria yang dominan di lokasi seperti tasik, sungai dan glasier dari semenanjung Fildes, Pulau King George. Dalam kajian ini, sepuluh sampel tanah dan sedimen telah dianalisis dengan menggunakan teknik PCR-DGGE berdasarkan gen 16S rRNA. Sebanyak 0.12-4.80 µg DNA telah berjaya diekstrak daripada satu gram tanah. Produk PCR daripada gen 16S rRNA diamplifikasi dengan menggunakan primer GC357f dan 907r dan kemudian dipisahkan dengan DGGE. Dalam tindak balas berantai polimerase (PCR), kesan perencatan daripada bahan kontaminasi terhadap polimerase dapat dikurangkan dengan penambahan 10 µg/µl bovine serum albumin (BSA) ataupun dengan pencairan DNA sebanyak 10-100 kali ganda. Semua sampel tanah mempunyai profil jalur DNA dominan yang berlainan. Jalur DNA dominan dipilih untuk penjujukan DNA dan seterusnya untuk identifikasi bakteria. Sebanyak 99 jalur dan 299 klon telah dipilih dari sepuluh lokasi: Tasik Antarctic (AL), Tasik GFZ (ZL), Tasik Estrellas (EL), Playa Elefantes (PE), Sungai Minas (MR), Glasier Collins (CG), Tasik Ketish (KL), Tasik Belen (BL), Tasik Geografos (GL) dan kawasan tangki minyak (OT). Jujukan-jujukan DNA dikategorikan kepada operasi unit taksonomi (OTUs) (kesamaan <97%) yang berlainan dan kemudian diklasifikasikan kepada 13 divisi bakteria iaitu Bacteroidetes (27.4%), Proteobacteria (25.7%), Acidobacteria (13.1%), Gemmatimonadetes (4.0%), Firmicutes (4.0%), Actinobacteria (3.4%), Chloroflexi (1.7%), Nitrospira (1.1%), Cyanobacteria (1.1%), WS3 (1.1%), Deionococcus-Thermus (0.6%), Spirochaetes (0.6%) dan BRC1 (0.6%). Sebanyak 15.4% jujukan DNA adalah bakteria yang masih belum dapat diklasifikasikan kepada divisi bakteria yang sedia ada. Hampir 90% daripada OTUs mempunyai skor identiti yang berdekatan dengan bakteria yang tidak dapat dikulturkan dalam pangkalan data NCBI. Sekitar 79% daripada OTUs pernah ditemui di luar kawasan benua Antarctica. Keanekaragaman bakteria di tanah yang menpunyai pH asid adalah lebih rendah. Sebagai contoh, PE dan OT (pH tanah adalah asid lemah) menunjukkan indeks keanekaragaman Shannon yang paling rendah. Secara keseluruhannya, tidak ada korelasi yang jelas dalam perubahan komuniti bakteria di kawasan yang dipengaruhi oleh aktiviti manusia kecuali di kawasan tangki minyak. Beberapa phylotypes yang pernah menujukkan kebolehan degradasi hidro-karbon telah ditemui di kawasan tersebut.

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LIST OF ABBREVIATIONS

ARISA	-	Automated ribosomal intergenic spacer analysis
BLAST	-	Basic local alignment search tool
bp	-	Base pair
BP	-	Before present
BSA	-	Bovine serum albumin
ca.	-	Approximately
CFB	-	Cytophaga-Flavobacteria-Bacteroidetes
cm	-	centimeter
CTAB	-	Cetyl Trimethyl Ammonium Bromide
DGGE	-	Denaturing gradient gel electrophoresis
DNA	-	Deoxyribonucleic acid
dNTP	-	2'-deoxyribonucleoside-5'-triphosphates
EDTA	-	Ethylenediaminetetraacetic acid
g	-	gram
ITS	-	Intergenic spacer
Kbp	-	Kilo base pair
km	-	kilometer
LSU	-	Large subunit
m	-	meter
M	-	Molar
Ma	-	Millions of years ago
$MgCl_2$	-	Magnesium chloride
MEGA	-	Molecular Evolutionary Genetic Analysis
mg	-	milligram
ml	-	milliliter
mm	-	milimeter
mM	-	milli molar
NaCl	-	Sodium chloride
NJ	-	Neighbor Joining

OTUs	-	Operational taxonomical units
PCR	-	Polymerase Chain Reaction
RDP	-	Ribosomal Database Project
rDNA	-	Ribosomal deoxyribonucleic acid
rRNA	-	Ribosomal ribonucleic acid
rpm	-	revolutions per minute
SDS	-	Sodium dodecyl sulfate
SSCP	-	Single-stranded conformation polymorphism
SSU	-	Small subunit
TAE	-	Tris-acetate-EDTA
TGGE	-	Temperature gradient gel electrophoresis
TRFLP	-	Terminal restriction fragment length polymorphism
Tris-HCl	-	Tris (hydroxylmethyl) aminomethane – hydroxychloride
U	-	Unit
UPGMA	-	Unweighted pair group method with arithmetic mean
V	-	Volt
VBNC	-	Viable but non-culturable
w/v	-	Weight per volume
µl	-	microliter
%	-	Percentage
°C	-	Degree Celsius
^{14}C	-	Carbon-14 (radiocarbon)

CHAPTER 1

INTRODUCTION

1.1 Background

Soil and sediment are considered as highly complex natural environments that contain large number of microorganisms with various kinds of species including prokaryotes and eukaryotes. Soil and sediment bacterial diversity, however, has long been recognized as a 'black box' until the late of 1980s. In general, most microbiologists believed that culturable bacteria only constituted about 1-10% of the total soil bacterial community. This is due to the limitations of conventional laboratory techniques in isolating and culturing bacteria from their native habitats into artificial environments.

Notable achievement and advances in the field of molecular biology since the discovery of nucleic acids structure by Watson and Crick in 1950s has led a significant revolution in the approaches to study microorganisms. For the past 20 years, knowledge on soil bacterial diversity is increasing gradually with the introduction of novel molecular tools such as total soil DNA extraction (metagenome), PCR amplification of the 16S rRNA gene (molecular marker for bacterial and archaeal identification), DNA fingerprinting, cloning and DNA sequencing technologies. For instance, Woese and Fox (1977) had found out that the small-subunit (SSU) ribosomal RNA was a suitable phylogenetic marker to determine evolutionary relationships between organisms. Subsequently, in the mid of 1980s, Pace and colleagues (1986) pioneered studies of microbial communities from environments without the need to cultivate them.

Since then, microbial ecologists had contributed a lot of effort to discover those viable but non-culturable (VBNC) bacteria that exist in the surrounding environment. Some of the recently published articles which concerned about soil metagenome clone libraries analyses had summarized the dominant groups of bacteria or phyla that were

commonly found in the soils. There are estimated to be at least 50 phyla within the bacterial kingdom. Almost half of them are based on uncultured bacteria from environmental cloned sequences and therefore are known as 'candidate' phyla. Overall, members from the phylum *Proteobacteria* and *Acidobacteria* were the most abundant and widely distributed in the soils at various parts of the world (Keller and Zengler, 2004; Janssen, 2006).

In this study, Antarctic bacterial diversity is investigated on soil and sediment samples from lakes, river and glacier of the Fildes Peninsula, King George Island. It is part of the South Shetland Islands and in the proximity of Antarctic Peninsula. As we know, Antarctica is extremely cold (average temperature 0°C to -50°C) and unfavorable for higher organisms such as plants and animals. However, previous studies have shown that prokaryotes diversity was surprisingly diverse on this continent (Bowman *et al.*, 2000; Brambilla *et al.*, 2001; Sjöling and Cowan, 2003). Besides, the environment in Antarctica is considered pristine because it was isolated geographically from other parts of the world since a long time ago. Furthermore, to date, there are not many reports on the diversity of bacteria in Antarctica especially in King George Island.

Antarctica is an attractive sampling location for microbiologists because it has a unique biodiversity and also a relatively simple microbial community structure due to its extreme climate, pristine and less accessibility from outsiders. Microorganisms especially psychrophiles and psychrotrophs have evolved with special kind of cold-adapted strategies in order to survive in such harsh and extreme environments. Therefore, this study may unveil novel bacteria or even taxa endemic to Antarctica.

Fildes Peninsula of King George Island has a relatively high anthropogenic influence with more than ten scientific research stations established by various countries such as Argentina, Brazil, Chile, China, Ecuador, South Korea, Peru, Poland, Russia and Uruguay. Human activities in Antarctica would have large impact on the endemic bacterial communities especially in some of the lakes near stations suspected of having perturbed

bacterial diversity. Therefore, soil and sediment samples from the lakes in the vicinity of Fildes Peninsula were subjected to PCR-DGGE analysis to compare different composition and evaluate the effect of human activities on the dominant bacterial community.

Nevertheless, microbial endemism in Antarctica is still an area of debate among researchers and may not be true in coastal areas of the continent especially near the Antarctic Peninsula and its adjacent islands where seasonal animal migration, long range transportation of pollutants and human inhabitation have occurred as compared to the internal part of the continent. Sequence data collected from this study would become valuable for comparison with other studies (deposited in NCBI GenBank database) to verify the phenomenon of microbial endemism in Antarctica.

1.2 Objectives

The objectives of this study are:

i) To compare composition of bacterial communities in the soils and sediments samples from the vicinity of Fildes Peninsula, King George Island based on 16S rDNA-DGGE banding profiles

ii) To identify dominant bacterial groups and compare their relative abundance in each location based on the partial 16S rDNA sequences

iii) To evaluate the taxonomical relationship of the bacterial communities

iv) To investigate the phenomena of bacterial endemism or cosmopolitanism in Fildes Peninsula, King George Island (maritime Antarctic)

CHAPTER 2

LITERATURE REVIEW

2.1 Antarctica

Antarctica is the fifth largest of the seven continents on Earth, approximately four million square kilometers and situated over the South Pole (Antarctic Circle). It contains some of the most extreme habitats on Earth, where it is the coldest, windiest, driest and highest continent. Antarctica contains roughly 70% of the world's fresh water, 90% of the world's ice (Ugolini and Bockheim, 2008), approximately 0.32% (45, 000 km^2; south of 60°S) of the continent is ice-free (Navas *et al.*, 2008; Ugolini and Bockheim, 2008). Due to its altitude and geographic position, Antarctica exerts a predominant influence on the southern hemisphere and global atmospheric and cryospheric systems (Ugolini and Bockheim, 2008). Ice-shelves have collapsed, releasing huge amount of water and thus increasing the sea level in consequences (Friedman, 1993; Mckay *et al.*, 1993).

Large ice sheets were present on parts of the Antarctica since 34 Ma and they have undergone repeated cycles of advance and retreat (Hambrey and Barrett, 1993; Zachos *et al.*, 2001; Johnson *et al.*, 2009). Nevertheless, there are some parts of Antarctica were escaped from ice cover during glacial periods. Antarctica consists of two main areas that are East Antarctica and West Antarctica. These two areas are separated by Transantarctic Mountains. During the summer, average temperature at South Pole is -27.5°C and fall drastically to -60°C during winter. The lowest temperature ever recorded was -89.2°C at the Russian Vostok station. Most of the time, the continent is also buffeted by strong winds with average wind speed 37 km/h. Antarctica is also the highest continent on Earth with an average elevation of 2,300 m above sea level.

Generally, there are three climatic regions in Antarctic continent and three major soil zones are existed. 1) Interior part of the continent is extremely cold with little snowfall.

2) Coastal areas have milder temperature and higher precipitation rates. 3) Antarctic Peninsula has a warmer and wetter climate. Antarctica is like a dry desert where the interior of the continent has an average annual precipitation (in the form of snow) only about 50 mm. This figure increases toward the coast but is still limited about 200 mm (Paul Ward and CoolAntarctica.com, http://www.coolantarctica.com/Antarctica%20fact%20file/antarctica%20environment/whats%20it%20like%20in%20Antarctica.htm).

There are three widely recognized biogeographical zones within Antarctica which are sub-, maritime, and continental Antarctic (Figure 2.1) (Convey, 2010). Antarctica is considered as the largest and most pristine wilderness on Earth due to its extreme climate and human-hostile environment. Antarctic ecosystems have limited trophic complexity, where penguins, seals, whale, fish, krill and sea birds are the vertebrate animal species could be found in Antarctica. There are only a few kinds of invertebrate insects able to survive in the terrestrial habitats such as mites, ticks and nematode worms. Vegetation is limited to species such as lichens, mosses and algae. Lichens are the dominant plant species that can be found in soils and rock surfaces (Ochyra *et al.*, 2008; Convey, 2010).

In contrast, prokaryotes are the dominant group of organism in the Antarctica and they play important roles in consumption chains, decomposition, nutrient cycling and biogeochemical cycles (Franzmann *et al.*, 1997; Yergeau *et al.*, 2009). Antarctic soils are highly aerobic and therefore anaerobic bacteria are rare to be found. Fungi (yeast, yeast-like, and filamentous species) have been identified in most of the Antarctic habitats but in low abundance (Vishniac, 1996).

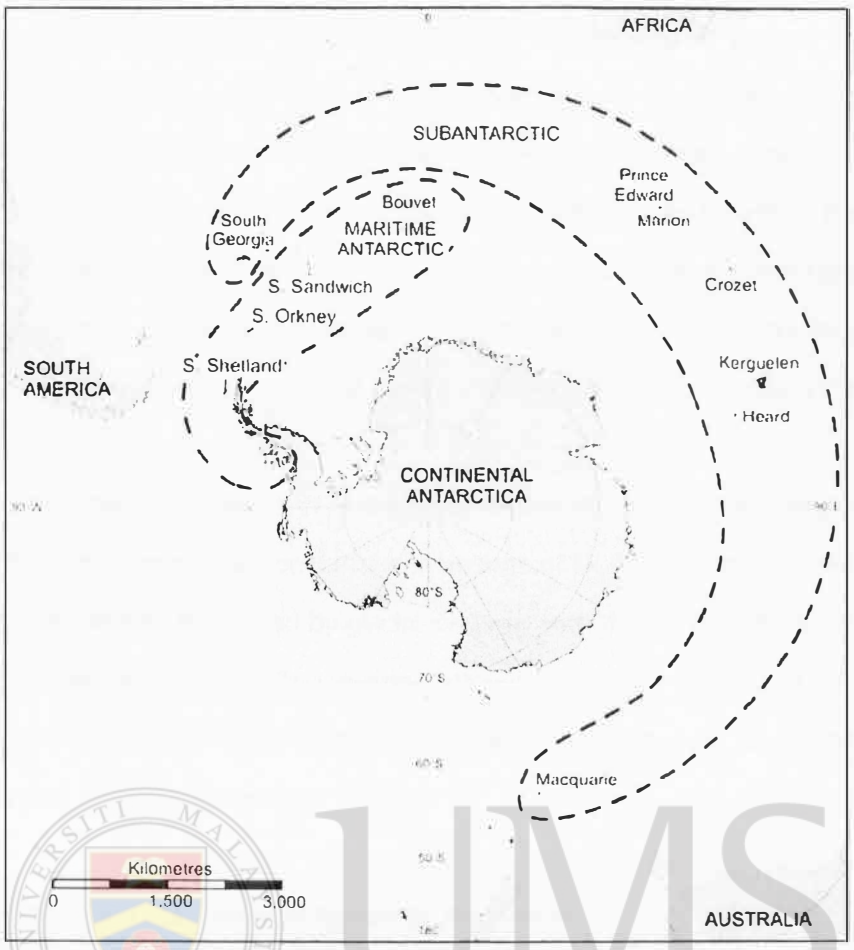

Figure 2.1: Map of Antarctica. (Convey, 2010).

2.1.1 Terrestrial Antarctica (Continent)

McMurdo Dry Valleys (MDVs) in South Victoria Land, Antarctica is the largest ice-free area (15%; 6692 km^2) and also the highest in elevation in the continent (Solopov, 1969; McKay *et al.*, 1993; Ugolini and Bockheim, 2008). Rapid change in temperature (around 15°C) and limited water availability had created extreme conditions, which is similar to the environmental conditions on Mars (McKay, 1993). In this situation, cryptoendolithic communities exist in the pore spaces of exposed rocks that offer protections from the desiccating surface (De La Torre *et al.*, 2003). Dry Valley mineral gravels may harbor quite a number of prokaryotes ranging from 10^6 to 10^8 cells per gram (Cowan *et al.*, 2002). Rapid freeze-thaw cycles can be lethal and microorganisms need to be well adapted to prevailing conditions (Russell, 2006).

The Schirmacher Oasis (70°45'1" S; 11°46' E), located in Queen Maud Land (Dakshin Gangotri Hill Ranges), East Antarctica is another ice-free area (35 km^2). This ice-free plateau is on average 100 meters above sea level and contains a number of small ponds and about 20 lakes (Walton, 1984). These lakes are 80 to 100 km from the sea and thus contain very low level of mineralization compared to saline lakes in the coastal areas (Shivaji *et al.*, 1989). Oases are as cold as the dry valleys. However they are covered with ice only during the Antarctic winter and have significant precipitation (Walton, 1984).

Another interesting place in Antarctic continent is Lake Vostok. Subglacial Lake Vostok is the eighth largest lake on Earth with an area of 14,000 km^2 and volume of 5,600 km^3. On top of the lake is covered by glacial ice layer with thickness of 4 km (Masalov *et al.*, 2001; Siegert *et al.*, 2001). The average temperature inside the lake is around -2°C and pressures could approach 400 atmospheres with a high oxygen level. The nutrient level is low and it is completely dark (D'Elia et al., 2008).

2.1.2 Maritime Antarctic and Antarctic Peninsula

Maritime Antarctica, which included Antarctic Peninsula and sub-Antarctic islands, has the warmest and most moist climate conditions in Antarctica. This included South Orkney Islands, South Sandwich Islands, South Shetland Islands, western coastal fringe of the Antarctic Peninsula south to the Marguerite Bay, Bouvetoya and Peter I Oy (Spaull, 1973). Antarctic Peninsula ice sheet is part of the marine-based West Antarctica Ice Sheet (Ingólfsson *et al.*, 1998). Most of the present rocky coastline of Antarctica probably started to become ice-free around 6000 ^{14}C years BP and soon after followed by the occurrence of lakes. In Antarctic Peninsula region, deglaciation begun about 5400-6300 ^{14}C years BP (Zale and Karlén, 1989). Deglaciation was completed by 6000 year BP on King George Island (Martinez-Macchiavello *et al.*, 1996).

The formation of soils in these regions was believed to have started since ca. 9500-6000 year BP. During the summer, there is a pronounced thaw period and soil developed more rapidly than other regions in Antarctica where less than 50 cm depth of

soils were thawed (Navas *et al.*, 2008). In Antarctic Peninsula, coastal water temperatures remains at -1.8°C for most of the year and never exceed 2°C (Grzymski *et al.*, 2006). Most of the time, soil temperature in most of the Antarctic areas is seldom rises above 10°C. However it may increase as high as 20-25°C during the summer (O'Brien *et al.*, 2004).

Soil water contents and organic matter accumulation were relatively high in some areas of the Antarctic Peninsula compared with interior Antarctica. Ice-cemented permafrost with an active layer of about 30 cm was found in South Shetland Islands (Serrano *et al.*, 1996; Serrano and López-Martínez, 2000) and during the summer this active layer would melt and interact with the upper part of permafrost (Bockheim, 1995). There is wide range of freshwater lakes in Antarctica which are characterized by short food chains dominated by microbes (Ellis-Evans, 1996).

2.2 Soil and Sediment Microbial Diversity

Microorganisms include prokaryotes, viruses, viroids, filamentous fungi, yeast, microalgae and protozoans. They are the most abundant organisms on Earth (Olembo, 1991). They are ubiquitous and survive in almost all climate areas including those once considered hostile to life such as the cold of Arctic and Antarctic, the heat of geysers and oceanic hot vents, and even deep within rocks (Colwell, 1997). Diversity of microbes may far exceed the current diversity of eukaryotic organisms (Torsvik and Øvreås, 2002).

Soil and sediment microorganisms are extremely diverse and complex especially in tropical and temperate regions (Kuske *et al.*, 1997). In general, the soil microbial community consists of members of the three domains of life; *Bacteria*, *Archaea* and *Eukarya*. They contribute up to 90% of the total biomass of soil microbiota and microfauna (Metting, 1993). Soil microbial communities are responsible for the soil respiration, decomposition, macro- and micro- nutrients uptake and fixation, detoxification of heavy metals, and also act as global carbon sinks (Schlesinger, 1991; Beare *et al.*, 1995). Around 10 billion microorganisms could be found in one gram of soil and it possibly constituted thousands different species (Roselló-Mora and Amann, 2001).

Less than 1% of the microorganisms have been successfully isolated in the culture media and observed under microscope. Therefore, soil microbial diversity to a large extent still remains unexplored.

Members from the domain Bacteria were the major group of organisms in soils, with approximately 10^9 bacterial cells per gram of soil (Torsvik *et al.*, 1990a; Dunbar *et al.*, 2002; Tringe *et al.*, 2005). The number of bacterial species in 100 g of soil and sediments are estimated to be around 0.5 to 1 x 10^6 based on DNA reassociation kinetics and 16S rRNA gene sequence similarities (Dykhuizen, 1998; Dunbar *et al.*, 1999) or roughly about 6000 different bacterial genome in one gram of soil considering the genome size of *Escherichia coli* as a unit (Torsvik *et al.*, 1996; Kuske *et al.*, 1997; Pace, 1997, 1999). Curtis *et al* (2002) also found that there could be around 7000 species in one gram of soil based on published data on ribosomal RNA gene based cloned libraries.

Soil and sediment consist of a variety of microhabitats with different physicochemical gradients. Soil heterogeneity has influenced on the spatial distribution of microorganisms (Trevors, 1998). Environmental conditions are discontinuous and thus microorganisms had adapted to certain microhabitats and live together in consortia. In other words, microorganisms are highly accumulated together in certain clumps or "hot spots" and they can be differed even over a small distance (van Elsas and van Overbeek, 1993). They interact with each other and also with other parts of the soil biota (Tiedje *et al.*, 2001). Factors such as mineral composition, salinity, pH, organic input, temperature, nutrient and water content determine which ecological niches would be available within the soil are (van Elsas *et al.*, 1997).

Diversity of bacteria from Antarctic soil and sediment had been previously reported and in general they were categorized into a few common bacterial phyla such as Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Planctomycetes, Proteobacteria, Verrucomicrobia and Spirochaetes (Smith *et al.*, 2000, 2006; Brambilla *et al.*, 2001; Bowman and McCuaig 2003;

Sjöling and Cowan, 2003; Saul *et al.*, 2005; Aislabie *et al.*, 2006, 2008; Li *et al.*, 2006; Xiao *et al.,* 2007; Chong *et al.*, 2009a, 2009b).

2.2.1 Measuring Soil and Sediment Microbial Diversity

Microbial diversity studies were generally interested on observing relative diversities of communities due to differences in stress, disturbance or other biotic or abiotic factors (Hughes *et al.*, 2001). Measurement of species diversity consists of two components, namely, species richness and species evenness (Trevors, 1998; Ravenschlag *et al.*, 1999; Øvreås, 2000). It consists of genetic variability within taxon (species), the number (richness) and relative abundance (evenness) of taxons and functional groups (guilds) in a community (Torsvik and Øvreås, 2002). Due to the dynamic nature of the microbial world, a community did not have a characteristic diversity. Instead it would change as the environment conditions shifted (Brock, 1987).

Shannon index (also sometime referred to Shannon-Wiener index or Shannon-Weaver index) is one of several diversity indices commonly used to measure biodiversity (Shannon and Weaver, 1971; Edwards *et al.*, 2001; Hill *et al.*, 2003). It is a non-parametric diversity index where it combines estimates of richness (number of species) and the evenness of the species in a population (Begon *et al.*, 1996; Yu *et al.*, 2006). The value will increase when there are more unique species or higher species evenness observed in a community (Whittaker, 1972; Johnson *et al.*, 2004). Typically, the value lies between 1.5 and 3.5 for ecological data and seldom exceeds 4.0 (Seaby and Henderson, 2006).

Traditional definition for species is applicable for higher organisms such as plants and animals but it is not well suited for prokaryotes or asexual organisms (Godfray and Lawton, 2001; Kirk *et al.*, 2004). The concept of bacterial species is rather complicated due to the plasticity of the bacterial genetics. For instance, DNA can be transferred into bacteria through plasmids, bacteriophages and transponsons (Kirk *et al.*, 2004). There is no official species definition for bacteria (Colwell *et al.*, 1995). In molecular aspect,