COMPOSITIONAL SHIFTS IN SOIL BACTERIAL COMMUNITIES FROM POLAR AND TROPICAL REGIONS IN RESPONSE TO SIMULATED WARMING

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DECLARATION

I declare that the material in this thesis is my own work except for quotations, excerpts, equations, summaries and references, which have been cited.

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ABSTRACT

The effects of global warming are increasing evidently in various biomes, and it is expected that soil bacterial diversity will be affected as they adapt towards higher environmental temperatures. However, the extent of changes may differ between distinct environments, such as between the tropical and polar region. This study aims to investigate the effects of simulated warming on bacterial diversity in soils from Kota Kinabalu (KK), Sabah and South Shetland Islands, Antarctica. Simulated warming was carried out using open-top chambers (OTC) at three plots in Kota Kinabalu for one year, while soils from two South Shetland Islands, specifically King George Island (KGI) and Deception Island (DCI), were placed in a growth chamber that simulates tropical-like conditions based on climate data obtained from Sabah Meteorological Department. Changes in bacterial diversity of tropical and polar soils in this study were monitored using V3-V4 16S rDNA amplicon sequencing. Optimization of DNA extraction method was first carried out using three different methods on ten soil samples collected around KK to avoid underestimation of soil bacterial diversity. The highest genomic DNA concentration was obtained using Method 1 (10.70 to 92.98 µg/g of soil), while DNA purity was highest for Method 3 with average A_{280}/A_{260} and A_{260}/A_{230} ratios of 1.91 and 1.76 respectively. Higher DNA purity achieved using Method 3 can be attributed to the use of silica spin columns to remove soil contaminants. Results from PCR-DGGE analysis showed that bacterial diversity in the soils from KK were underestimated using Method 1 and 2, mainly due to PCR inhibition by co-extracted contaminants such as humic acid. As for Method 3, total number of DGGE bands and Shannon-Weaver indices were the highest, which indicated that Method 3 gave the most accurate representation of soil bacterial diversity in KK soils. Method 3 was therefore the most suitable DNA extraction method for KK soils in this study. Simulated warming using OTCs on KK soils led to an average increase of 0.81 to 1.15 °C in treatment plots after 12 months. V3-V4 16S rDNA amplicon analysis showed that initial bacterial diversity in KK soils were predominated with Actinobacteria, Acidobacteria and Proteobacteria at phylum level; *Gaiella, Candidatus Koribacter* and *Candidatus Solibacter* spp. at genus level. Significant changes in relative abundance of bacterial phyla Bacteroidetes and Chloroflexi were detected after 12 months of simulated warming. Increases in relative abundance of Actinobacteria and Planctomycetes were also observed in treatment plots. Substantial changes were observed at genus level, where relative abundance of *Gaiella, Bradyrhizobium* and *Chthoniobacter* decreased substantially after 12 months of incubation. Substantial increase in relative abundance of *Bacillus* was also detected, where RT-PCR analysis confirmed the presence of pathogenic bacteria from this bacterial group in the KK soils. Initial diversity of soil bacteria phyla was determined for KGI and DCI soils, which mainly include Actinobacteria, Proteobacteria and Verrucomicrobia. Comparisons at genus level showed different compositions of bacteria between KGI and DCI soils, where

Gaiella spp. was predominant in KGI soils while bacterial genera in DCI soils were more evenly distributed. Initial DGGE analysis provided evidence that 12 months of simulated tropical-like conditions led to significant changes in overall bacterial diversity of KGI soils, but not for DCI soils. V3-V4 16S rDNA amplicon analysis showed that most bacterial phyla in both soils did not thrive after 12 months of simulated tropical-like conditions. There were however increases in relative abundance of Proteobacteria by more than 150 % in KGI soils, while a twofold increase in relative abundance was observed for Acidobacteria and Chloroflexi in DCI soils. Substantial changes in bacterial composition were also observed at genus level, where *Methylobacterium* spp. was most predominant in both soils after 12 months of incubation. The genus *Methylobacterium* may consist of opportunistic human pathogens, which warrants further monitoring. Increases in relative abundance of potentially pathogenic bacteria such as *Mycobacterium, Massilia* and *Williamsia* spp. were detected, and further validation will be required to determine pathogenicity of these bacterial genera, if any. Overall, the results of this study provided baseline diversity of soil bacteria at sites in Kota Kinabalu and South Shetland Islands. This study gave an indication of how soil bacteria from KK would respond towards soil warming in the coming decades, assisting in potential microbial conservation efforts and related future studies. The response of soil bacteria from KGI and DCI towards simulated tropical-like conditions also highlighted the importance of preventing accidental transfer of soils out of Antarctica.

ABSTRAK

PERGESERAN KOMPOSISI DI KOMUNITI BAKTERIA TANAH DARI KAWASAN KUTUB DAN TROPIKA SEBAGAI TINDAK BALAS TERHADAP PEMANASAN SIMULASI

Kesan pemanasan global semakin meningkat dalam pelbagai biom, dan kepelbagaian bakteria tanah dijangka akan terjejas apabila mereka menyesuaikan diri dengan suhu alam sekitar yang lebih tinggi. Walau bagaimanapun, kadar perubahan boleh berbeza antara persekitaran yang berlainan, seperti di antara kawasan tropika dan kutub. Kajian ini bertujuan untuk mengkaji kesan pemanasan simulasi terhadap kepelbagaian bakteria di tanah dari Kota Kinabalu (KK), Sabah dan Kepulauan Shetland Selatan, Antartika. Pemanasan simulasi dilakukan dengan menggunakan ruang terbuka (OTC) di tiga petak dari Kota Kinabalu selama satu tahun, manakala tanah dari dua Kepulauan Shetland Selatan, khususnya Pulau King George (KGI) dan Pulau Deception (DCI), diletakkan dalam bilik pertumbuhan yang mensimulasikan keadaan tropika berdasarkan data iklim yang diperoleh dari Jabatan Meteorologi Sabah. Perubahan kepelbagaian bakteria tanah tropika dan kutub dalam kajian ini dipantau menggunakan penjujukan amplikon V3-V4 16S rDNA. Pengoptimuman kaedah pengekstrakan DNA mula-mula dijalankan dengan menggunakan tiga kaedah yang berbeza atas sepuluh sampel tanah yang dikumpul sekitar KK untuk mengelakkan pengurangan kepelbagaian bakteria tanah. Kadar kepekatan DNA genomik tertinggi diperoleh dengan menggunakan Kaedah 1 (10.70 hingga 92.98 µg/g tanah), manakala ketulenan DNA adalah tertinggi untuk Kaedah 3 dengan nisbah purata A_{280}/A_{260} dan A_{260}/A_{230} masing-masing 1.91 dan 1.76. Ketulenan DNA lebih tinggi yang dicapai dengan Kaedah 3 dapat dikaitkan dengan penggunaan lajur putaran silika untuk menghilangkan bahan cemar tanah. Hasil daripada analisis PCR-DGGE menunjukkan bahawa kepelbagaian bakteria di tanah KK dikurangkan dengan Kaedah 1 dan 2, terutamanya disebabkan perencatan PCR oleh kontaminan yang diekstrak seperti asid humat. Bagi Kaedah 3, jumlah bilangan band DGGE dan indeks Shannon-Weaver adalah tertinggi, ini menunjukkan bahawa Kaedah 3 memberi gambaran yang paling tepat mengenai kepelbagaian bakteria tanah di tanah KK. Oleh itu kaedah 3 adalah kaedah pengekstrakan DNA yang paling sesuai untuk tanah KK dalam kajian ini. Pemanasan simulasi menggunakan OTC pada tanah KK menyebabkan peningkatan purata sebanyak 0.81 hingga 1.15 °C dalam OTC selepas 12 bulan. Analisis amplikon V3-V4 16S rDNA menunjukkan bahawa kepelbagaian bakteria di tanah KK didominasi dengan Actinobacteria, Acidobacteria dan Proteobacteria pada peringkat filum; Gaiella, Candidatus Koribacter dan Candidatus Solibacter spp. pada peringkat genus. Perubahan yang ketara dalam jumlah relatif bakteria filum Bacteroidetes and Chloroflexi dikesan selepas 12 bulan pemanasan simulasi. Peningkatan jumlah relatif Actinobacteria dan Planctomycetes juga diperhatikan dalam OTC. Perubahan besar diperhatikan pada peringkat genus, di mana jumlah relatif Gaiella,

Bradyrhizobium dan Chthoniobacter berkurangan secara mendadak selepas 12 bulan. Peningkatan besar dalam jumlah relatif Bacillus juga dikesan, di mana analisis RT-PCR mengesahkan kehadiran bakteria patogen dari kumpulan bakteria ini di tanah KK. Kepelbagaian awal bakteria tanah filum ditentukan untuk tanah KGI dan DCI, kebanyakan terdiri dari Actinobacteria, Proteobacteria dan Verrucomicrobia. Perbandingan pada peringkat genus menunjukkan komposisi bakteria yang berlainan antara tanah KGI dan DCI, di mana Gaiella spp. paling dominan di tanah KGI manakala genus bakteria di tanah DCI lebih seimbang. Analisis DGGE awal membuktikan bahawa inkubasi dalam keadaan tropika selama 12 bulan menyebabkan perubahan ketara dalam keseluruhan kepelbagaian bakteria tanah KGI, tetapi bukan untuk tanah DCI. Analisis amplikon V3-V4 16S rDNA menunjukkan bahawa kebanyakan filum bakteria di kedua-dua tanah tidak dapat menyesuaikan diri selepas inkubasi dalam keadaan tropika selama 12 bulan. Walau bagaimanapun, terdapat peningkatan jumlah relatif Proteobakteria yang lebih daripada 150% di tanah KGI, manakala peningkatan dua kali ganda dalam jumlah relatif diperhatikan untuk Acidobacteria dan Chloroflexi di tanah DCI. Perubahan besar dalam komposisi bakteria juga diperhatikan pada peringkat genus, di mana Methylobacterium spp. paling dominan di kedua-dua tanah selepas 12 bulan pengeraman. Genus Methylobacterium mungkin terdiri daripada patogen manusia oportunis, dan pemantauan lebih lanjut diperlukan. Peningkatan jumlah relatif bakteria patogen yang berpotensi seperti Mycobacterium, Massilia dan Williamsia spp. telah dikesan, dan pengesahan selanjutnya diperlukan untuk menentukan patogenisiti genus bakteria ini, jika ada. Secara keseluruhannya, hasil kajian ini mendedahkan kepelbagaian bakteria tanah di tapak di Kota Kinabalu dan Kepulauan Shetland Selatan. Kajian ini memberi petunjuk tentang bagaimana bakteria tanah dari KK akan bertindak balas terhadap pemanasan tanah dalam beberapa dekad akan datang, membantu dalam usaha pemuliharaan mikrob dan kajian masa depan yang berkaitan. Tindak balas bakteria tanah dari KGI dan DCI terhadap inkubasi dalam keadaan tropika juga menekankan kepentingan pencegahan pemindahan tanah secara tidak sengaja dari Antartika.

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℃	-	degree Celsius
µg	-	microgram
µm	-	micrometre
µM	-	micromolar
µL	-	microlitre
χ^2	-	Chi-square
η_p^2	-	Partial Eta squared
ANOVA	-	analysis of variance
atpE	-	atp synthase epsilon chain
BLAST	-	basic local alignment search tool
bp	-	base pair
cDNA	-	complementary DNA
cer	-	cereulide
cm	-	centimetre
C	-	carbon
CO_2	-	carbon dioxide
CTAB	-	cetyltrimethylammonium-bromide
DCI	-	Deception Island
DGGE	-	denaturing gradient gel electrophoresis
DNA	-	deoxyribonucleic acid
dNTP	-	deoxynucleotide triphosphate
g	-	gram
Gb	-	gigabytes
kb	-	kilo base pair
km^2	-	square kilometre
KGI	-	King George Island
LCA	-	lowest common ancestor
min	-	minute
mL	-	millilitre
mm	-	millimetre

mM	-	millimolar
M	-	molar
$MgCl_2$	-	magnesium chloride
N	-	nitrogen
NaCl	-	sodium chloride
NCBI	-	National Center for Biotechnology Information
NGS	-	next generation sequencing
ompA	-	outer membrane protein A
OTC	-	open top chamber
ppm	-	parts per million
P^t	-	p value for paired t-test
PCR	-	polymerase chain reaction
PEG	-	polyethylene glycol
RNA	-	ribonucleic acid
rcf	-	relative centrifugal force
rDNA	-	ribosomal DNA
rRNA	-	ribosomal RNA
RT-PCR	-	reverse transcription polymerase chain reaction
sec	-	second
spp.	-	species
SD	-	standard deviation
SDS	-	sodium dodecyl sulfate
TAE	-	Tris–acetate-EDTA
TE	-	Tris EDTA
UPGMA	-	unweighted pair group method with arithmetic mean
V	-	volt
vol/vol	-	volume by volume
w/v	-	weight by volume

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The impact of global warming is increasingly evident in various biomes (Walther *et al.*, 2002), where increases in global surface temperatures have reached 1.0 °C above pre-industrial levels (Stocker *et al.,* 2014). Further increases in global surface temperatures are expected in the coming decades, and this can be attributed to increased concentrations of atmospheric carbon dioxide that has exceeded 410 ppm in 2019. It remains unclear how an increase in environmental temperature would affect terrestrial bacteria, which are involved in various environmental processes such as biogeochemical cycling (Lefcheck *et al.*, 2015; Walther *et al.*, 2002).

There is also increasing concern of possible associations between global warming and the emergence of bacterial infectious diseases, which has been observed in marine environments (Colwell, 1996). An increase of 1 °C in sea surface temperature in the Baltic Sea was reported to coincide with a twofold increase of observed *Vibrio* cases (Baker-Austin *et al.*, 2012). Increases in environmental temperature have also led to anthrax outbreak in the Yamal Peninsula, where permafrost containing anthrax spores was thawed due to temperature anomalies in the region (Golovnev, 2017). Currently, there is a lack of information on the effects of temperature rise on soilborne pathogenic bacteria, which stresses the importance of determining their presence in terrestrial environments and how elevated temperatures affect these bacteria. It is

hypothesized that pathogenic bacteria are present in soils from the tropics and maritime Antarctic, particularly in areas nearby of marine environments.

The composition and diversity of soil microbiota is dependent on local environmental conditions (Lindström & Langenheder, 2011). The maritime Antarctica and Sabah are two regions highly dissimilar in terms of biodiversity and environmental conditions. The climate in maritime Antarctica can be described as very cold with extremely dry conditions while the climate in Sabah is characterized by high temperatures and humidity throughout the year. There are currently few studies that have fully described the diversity of soil bacteria in both regions. With such contrasting environmental conditions, it is hypothesized that different composition of bacterial communities will be observed between soils from maritime Antarctica and the tropics.

In order to determine the effects of temperature rise on soil bacterial diversity and the possible surge of pathogenic bacteria in the tropics and maritime Antarctica, simulated warming will be carried out using open top chambers (OTC) and growth chambers respectively. OTCs are passive warming systems that are capable of simulating temperature rise of one to two degree celsius using solar energy as a heat source (Camac *et al.*, 2015; Bokhorst *et al.*, 2011; Hollister and Webber, 2000; Marion *et al.*, 1997; Yergeau *et al.*, 2012). Among the advantages of OTC include that it causes less environmental perturbations and more economical to maintain for longer periods. As for growth chambers, they are active warming systems that involve the control of energy flow to maintain constant heat differentials within an enclosed environment (Bárcenas-Moreno *et al.*, 2009; Yergeau and Kowalchuk, 2008). Growth chambers are able to simulate warming while keeping other environmental factors such as air humidity, water supply and photosynthetic active radiation constant throughout the duration of the experiment. Both OTCs and growth chambers are therefore viable options to carry out simulated warming for this study.

To determine the effects of simulated warming on soil microbes, changes in soil microbial community composition has to be monitored. The analysis of

microbial diversity in the environment was previously hindered due to the inability of most microbes to grow on conventional media. As such, new molecular-based analysis were established to identify microbes regardless of cultivability using denaturing gradient gel electrophoresis (DGGE) or NGS sequencing of the metagenome of samples collected from the environment. Both DGGE and NGS sequencing are molecular approaches that rely on the use of phylogenetic markers such as 16S rDNA to determine and monitor bacterial diversity. 16S rDNA is widely used as a phylogenetic marker because it can be found in all prokaryotes and has hypervariable regions as targets for microbial diversity studies (Case *et al.*, 2007; Janssen, 2006; Kolbert & Persing, 1999; Ludwig & Schleifer, 1994; Pereira *et al.*, 2010). The V3-V4 hypervariable region of 16S rDNA is widely used as a target for diversity studies because primers targeting this region showed less bias at phylum and genus levels, and higher coverage of genera can be achieved compared to other primers targeting other regions of 16S rRNA (Cai *et al.*, 2013; Chan *et al.*, 2015; Hong *et al.*, 2015; Klindworth *et al.*, 2013; Takahashi *et al.*, 2014).

To avoid biases in DNA-based diversity analysis of tropical soil microbes, the choice of DNA extraction method will be crucial. Currently, there is no standard DNA extraction protocol that is best suited for all tropical soil types. Previous studies have reported how different efficiencies in DNA extraction methods can affect microbial community profiles from soils (Carrigg *et al.,* 2007; de Lipthay *et al.,* 2004; Gupta *et al.,* 2017). The use of optimal DNA extraction methods is therefore prerequisite for an accurate representation of soil microbial communities, especially for tropical soils which are rich in bacterial diversity. For this study, three different DNA extraction methods were compared for tropical soils collected from Kota Kinabalu, Sabah to determine the most suitable extraction method for soils from this region. As for genomic DNA from the polar soils, they were extracted using a well established extraction method (Zhou *et al.*, 1996) used previously for soils from the same region.

The main aim of this study was to determine the baseline diversity of bacterial phyla and genera in soils from Kota Kinabalu, Sabah and South Shetland Islands, Antarctica. Tropical soils from Kota Kinabalu were then subjected to 12

months of *in situ* warming using OTCs to determine whether significant changes in abundance of bacterial phyla and genera will be observed. As for the polar soils from South Shetland Islands, a growth chamber was used to simulate 12 months of tropical-like conditions, specifically to mimic air temperature, humidity and daylight exposure observed in Kota Kinabalu, Sabah. The changes in abundance of bacterial phyla and genera in response to tropical-like conditions were monitored to determine the survivability of polar microbes when subjected to warmer climates. The results from this study will provide a better overview of soil bacterial composition from these regions and they adapt towards higher temperatures, assisting in soil bacterial management and related future studies.

1.2 Objectives

i) To determine the most suitable DNA extraction method for soils collected from Kota Kinabalu, Sabah.

ii) To determine the effects of *in situ* warming on the composition and relative abundance of bacterial phyla and genera in tropical soils collected from Kota Kinabalu, Sabah.

iii) To determine the presence of pathogenic bacteria *Bacillus cereus, Mycobacterium tuberculosis* and *Chlamydia pneumoniae* in tropical soils collected from Kota Kinabalu, Sabah using reverse transcription PCR.

iv) To determine the effects of simulated tropical-like conditions on the composition and relative abundance of bacterial phyla and genera in polar soils collected from South Shetland Islands, Antarctica.