Analysis of bacterial communities of King George and Deception Islands, Antarctica using high-throughput sequencing

C. Y. Chua[1], S. T. Yong[1], M. A. González[2], P. Lavin[2], Y. K. Cheah[3], G. Y. A. Tan[4] and C. M. V. L. Wong[1,4,*]

[1]Biotechnology Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia
[2]Instituto Antártico Chileno, Plaza Muñoz Gamero 1055, Punta Arenas, Chile
[3]Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
[4]National Antarctic Research Centre, University of Malaya, 50603 Kuala Lumpur, Malaysia

King George Island (KGI) and Deception Island (DCI) are members of the South Shetland Islands in Antarctica, each with their own landscape and local environmental factors. Both sites are suitable for long-term monitoring of bacterial diversity shift due to warming, as temperature rises relatively faster than East Antarctica. This study was conducted to determine and compare the baseline diversity of soil bacteria in KGI and DCI. 16S rDNA amplicons of bacteria from both sites were sequenced using Illumina next generation sequencer. Results showed that major phyla in KGI and DCI were Actinobacteria, Proteobacteria, Chloroflexi, Verrucomicrobia, Bacteriodetes and Acidobacteria. The distribution and evenness of the soil bacterial communities varied at genus level. The genera *Sphingomonas* sp. was predominant at both sites while the subsequent six major genera differed. Two bacterial genera, *Legionella* and *Clostridium* were also found in low abundance in both sites, both of which may contain pathogenic members. Further verification will be required to determine whether the pathogenic members of these genera are present in both sites.

Keywords: Antarctica, South Shetland Islands, soil bacterial diversity, 16S rDNA, pyrosequencing.

Introduction

ANTARCTICA represents one of the most extreme environments on earth, where aspects such as temperature, precipitation, humidity and nutrient availability are low[1]. Despite such hostile conditions, diverse microbial communities are able to thrive and form an integral part of terrestrial trophic interactions in Antarctica[2]. King George Island (KGI) and Deception Island (DCI) are members of the South Shetlands archipelago in Antarctica. KGI represents the largest of the South Shetland Islands (1310 sq. km) with a rich biodiversity and is strongly influenced by the local climate[3]. DCI is a caldera of an active strato volcano, that is significantly smaller (72 sq. km), with a unique landscape and environment. The thermal activities in DCI along with fluctuations in temperature gradient, oxygen level and other environmental conditions promote the proliferation of microorganisms with varying metabolic profiles[4]. On the other hand, an increase of human activities in KGI resulted in high concentrations of trace elements in soil samples[5]. It would therefore be interesting to determine whether the differences in local environmental factors between both islands would result in spatial heterogeneity of soil bacterial diversity[6].

The warming of Antarctic soils is affecting the microbial diversity and is expected to alter the diversity of bacteria in the terrestrial environment[7]. Currently, there is limited data on the profile of diversity. It is important to determine the baseline bacterial diversity to continue monitoring the effect of warming or anthropogenic influence at those sites. Both KGI and DCI are located in West Antarctica which is known to warm up relatively faster than East Antarctica. Both sites are therefore ideal for monitoring the current and future bacterial diversity shifts due to warming.

Studies on soil bacterial diversity were previously dependent on traditional culturing methods, which were inadequate and biased as only about 1% of bacteria can be cultured *in vitro*. The present molecular approaches, such as denaturing gradient gel electrophoresis (DGGE) and next-generation sequencing (NGS), target phylogenetic markers such as 16S rDNA allow a more comprehensive analysis of bacterial diversity. DNA amplification targeting the V3–V4 hypervariable region of 16S rDNA is commonly used for the analysis of bacterial communities, because this technique provides an overview of bacterial diversity in the samples[8].

The use of PCR-DGGE to analyse soil bacterial diversity has revealed similar richness[9,10]. However, the true

*For correspondence. (e-mail: michaelw@ums.edu.my)

extent of soil bacterial diversity in Antarctica, could not be fully elucidated through PCR-DGGE analysis as minor bacterial groups were most likely overlooked[11]. The use of NGS platforms such as Illumina MiSeq and Roche 454 allows higher throughput sequencing of DNA suitable for more in-depth analysis of soil bacterial diversity[7,11]. Therefore, this study aims to determine and compare the baseline soil bacterial diversity in KGI and DCI using the Illumina MiSeq NGS platform.

Present study

Soil samples were collected from KGI (S62°11′35.3″; W58°56′6.2″) and DCI (S62°59′023″; W60°40′51.7″), Antarctica in February 2007 and stored at –20°C immediately after sampling. Sampling sites in KGI and DCI are shown in Figures 1 and 2 respectively. The pH of soil samples as measured using a pH meter (pH 2700, Eutech Instruments) with a 1 : 5 soil-to-deionized water slurry ratio[12]. Total bacterial genomic DNA was extracted from soils using a previously described method[13]. The concentration and purity of the extracted genomic DNA were measured using the NanoDrop™ 2000 spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA).

The V3–V4 region of the bacterial 16S rRNA gene was amplified using locus-specific primers, S-DBact-0341-b-S-17 (5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3′) and S-D-Bact-0785-a-A-21 (5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3′)[8]. The 16S rDNA amplicons were subjected to paired-end sequencing on Illumina MiSeq platform using 2 × 250 bp MiSeq Reagent Kit v2 (Illumina® Inc., San Diego, California). Raw sequencing reads obtained for KGI and DCI soil samples were processed and assembled into single contigs using the make.contigs command of MOTHUR v.1.37.0 (ref. 14). The assembled contigs of both samples were aligned using Basic Local Alignment Search Tool (BLAST) against NCBI's nucleotide collection (nr/nt) database. The BLAST output files were imported into MEGAN5 software[15] for taxonomic classification using default LCA (lowest common ancestor) and analysis parameters. The GI mapping file used for taxonomic identification of sequencing reads was updated as of February 2016. The raw sequence reads for KGI and DCI samples are available at NCBI with accession numbers SRR5138461 and SRR138462 respectively.

Table 1. Soil bacterial DNA yield and purity

Soil sample	Genomic DNA concentration (μg/g of soil)	A_{260}/A_{280}	A_{260}/A_{230}
King George Island	34.3	1.90	2.17
Deception Island	6.1	1.91	2.30

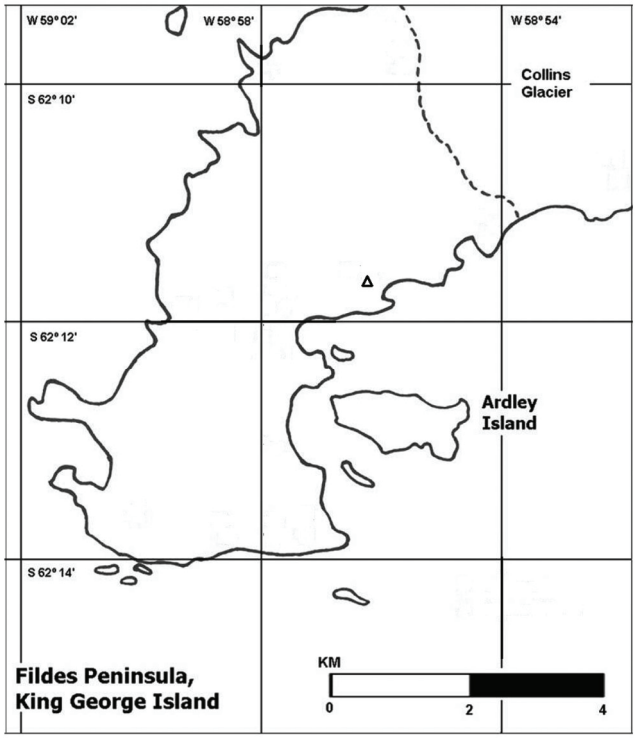

Figure 1. Soil sampling site at King George Island, Antarctica.

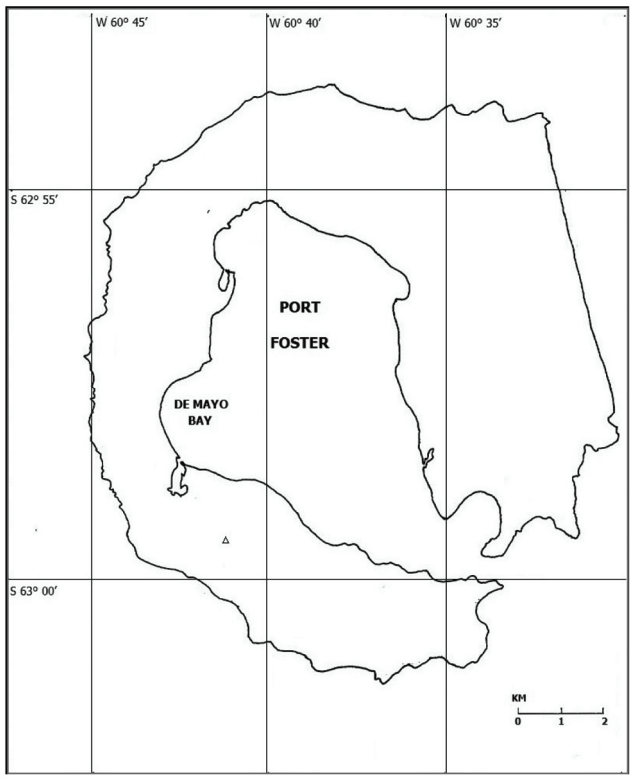

Figure 2. Soil sampling site at Deception Island, Antarctica.

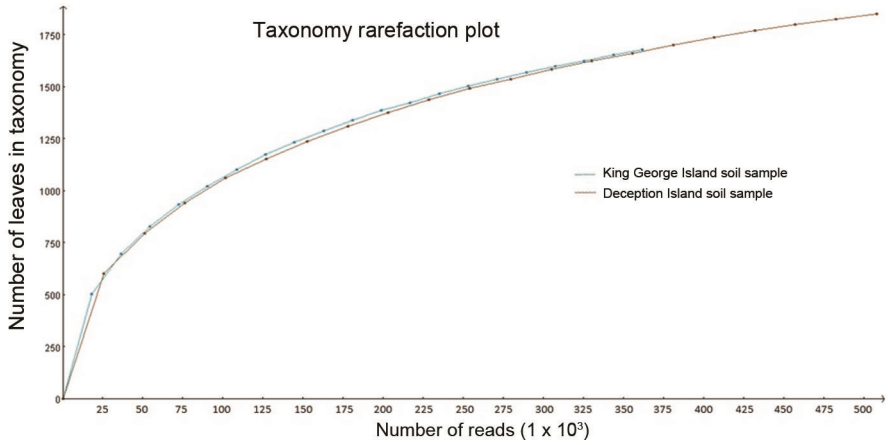

Figure 3. Rarefaction analysis of V3/V4 pyrosequencing reads of 16S rDNA amplicons in soil samples from King George Island and Deception Island.

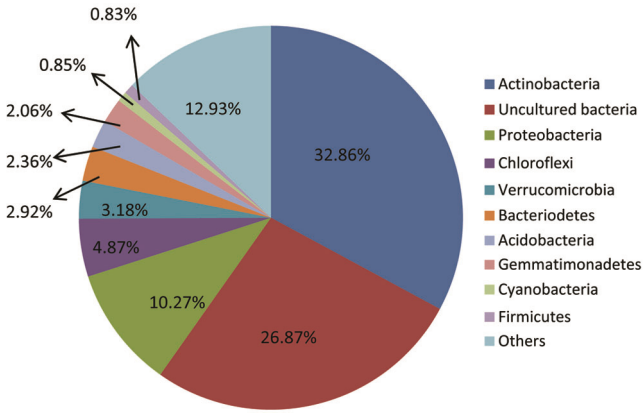

Figure 4. Major bacterial phyla found in soils from King George Island.

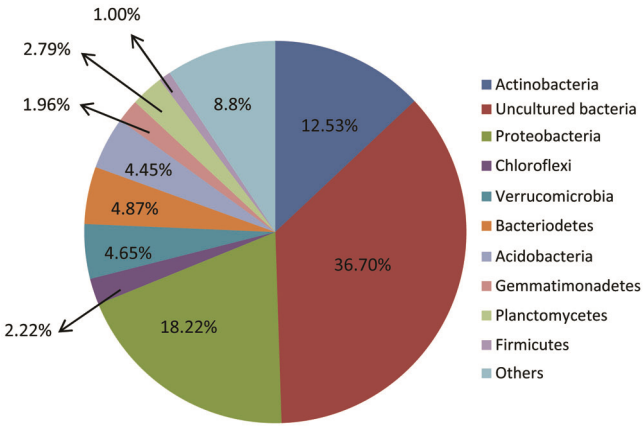

Figure 5. Major bacterial phyla found in soils from Deception Island.

Results and discussion

The pH values of KGI and DCI soil samples were 6.4 and 7.3 respectively. Bacterial genomic DNA yields from KGI and DCI soil samples were 34.3 µg/g and 6.1 µg/g respectively (Table 1). This was relatively higher than the yields obtained in previous studies[2,9]. The genomic DNA purity of both samples, based on the $A_{260/280}$ ratio, was within the desired range of 1.80–2.00 (Table 1), indicating that the extraction method used in the study[13] was suitable for soil samples.

The primer pair S-DBact-0341-b-S-17 and S-D-Bact-0785-a-A-21 was used in this study, because it has minimal bias and optimal overall coverage for PCR-based microbial diversity studies[8]. The assembled contigs of both samples were aligned against NCBI's nucleotide collection (nr/nt) database updated[15] on March 2016 (ref. 15). A total of 609,991 out of 626,552 reads (97.36%) of KGI, and 690,730 reads (99.73%) out of 692,534 reads of DCI had hits to sequences in NCBI nucleotide database. The total number of reads assigned to each bacterial taxon represented the number of times the same sequence was observed within the bacterial community. This allowed the estimation of relative abundance of each bacterial taxon classified in this study. The Shannon-Weaver indices of both KGI and DCI samples were calculated[15] and displayed similar values of 4.305 and 4.299 respectively. This showed that soil bacterial communities at both sites had similar levels of diversity. Rarefaction of the sequencing reads obtained for KGI and DCI was analysed using MEGAN5 software[15]. The rarefaction curves of both samples were reaching a plateau but did not achieve species saturation (Figure 3). Hence, the data generated gave a general overview of major bacterial phyla from the two sites. Nevertheless, the generated data had higher precision and gave a more accurate overview of bacterial diversity compared to DGGE, which relies on a limited number of representative 16S rDNA sequences chosen selectively.

Major bacterial phyla from the two sites in KGI and DCI are shown in Figures 4 and 5 respectively. The major phyla from both sites at KGI and DCI in descending order are Actinobacteria, Proteobacteria, Chloroflexi, Verrucomicrobia, Bacteriodetes and Acidobacteria. These

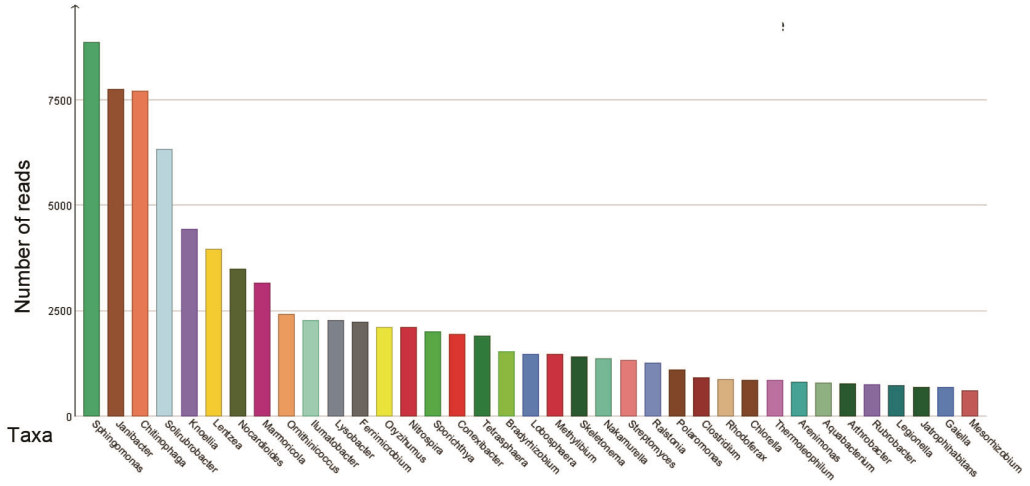

Figure 6. Bacterial genera found in soil sample from King George Island. Only taxa assigned with ≥0.5% of total reads were included in the analysis.

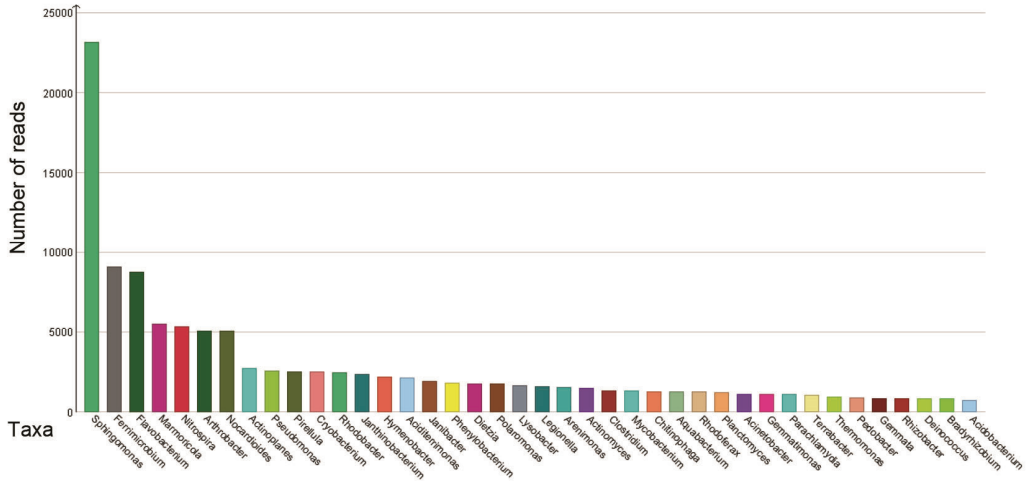

Figure 7. Bacterial genera found in soil sample from Deception Island. Only taxa assigned with ≥0.5% of total reads were included in the analysis.

phyla represent the major bacterial community of Antarctic soils which have adapted to the harsh and extreme environmental conditions[16]. The predominance of Actinobacteria in both Antarctic soils was expected, given their roles in essential ecological functions such as degradation of organic matter and maintenance of environmental stability[17]. The presence of Verrucomicrobia as a major bacterial phylum in both soils is in accordance with a previous claim that the relative abundance of Verrucomicrobia in soils was under-recognized while its predominance was evident across various biomes[18]. A substantial portion of the bacterial communities in both KGI (26.87%) and DCI (36.70%) soil samples were classified as uncultured. This emphasizes the need for efforts to characterize and identify these bacteria in order to understand their roles and functions.

At genus level, only 10.21% and 11.69% of the reads were classified for KGI and DCI samples respectively. This was expected because the targeted V3–V4 region of 16S rDNA is short and provides lower confidence for classification at lower taxonomic levels. Most of the bacterial genera were classified as uncultured bacteria that could only be identified at higher taxonomic levels. A total of 37 (Figure 6) and 40 (Figure 7) genera with relative abundance equal or more than 0.5% were found at KGI and DCI respectively. The overall composition of soil bacterial community in the KGI sample was more evenly distributed when compared to the DCI sample. The heterogeneity patterns in soil bacterial communities at lower taxonomic levels (genera level) are more obvious than those at higher taxonomic levels (phyla level), as bacterial species are more sensitive to spatial and environmental factors[16,19].

The six major genera in KGI were *Sphingomonas*, *Janibacter*, *Chitinophaga*, *Solirubrobacter*, *Knoellia* and *Lentizie*, while the major genera in DCI were *Sphingomonas*, *Ferrimicrobium*, *Flavobacterium*, *Marmoricola*,

Nitrospira and *Arthrobacter* (Figures 6 and 7). *Sphingomonas* sp. was common at both sites and its predominance was more apparent in DCI. This could be due to their ability to survive in low-nutrient environments inherent to Antarctica[16], which allows them to thrive better than other bacteria. Apart from that, differences in genera predominance at the two sites, were mostly due to local characteristics of the soils. Soil samples collected in KGI were in close proximity to a scientific station, and contain high levels of heavy metals such as Zn, Pb, Cd, Cr and Ni (ref. 5). This could have an impact on the relative abundance of certain bacterial genera, where the preliminary data showed the presence of *Janthino bacterium* in DCI but not in KGI. Lower relative abundance of *Arthrobacter* in KGI was also observed, while this group was the fifth most commonly identified bacterial genera in DCI. A recent study displayed the differential sensitivity of bacteria towards heavy metal concentrations, where *Arthobacter* and *Janthino bacterium* were more sensitive than *Geobacter* and *Fusibacter*[20]. The bacterial genera *Legionella* and *Clostridium* both sites, both of which may contain pathogenic members were also detected at both sites. But, their population was relatively low and insufficient to cause pathogenicity, if any.

The results of this study provide a baseline of soil bacterial diversity of KGI and DCI. Spatial heterogeneity of soil bacterial diversity between both sites was observed at the genus level. Any variations between soil bacterial communities are to be observed at lower taxonomic levels and these data can be obtained using NGS. NGS sequencing will be important in monitoring diversity change due to warming as the exercise requires precision, which cannot be achieved using DGGE analysis. In future studies, it will be interesting to monitor how the major genera at both sites such as *Sphingomonas* sp. and minor genera with members that may be pathogenic, such as *Legionella* and *Clostridium* are affected by a rise in temperature.

1. Wynn-Williams, D. D., Ecological aspects of Antarctic microbiology. *Adv. Microb. Ecol*., 1990, **11**, 71–146.
2. Smith, J. J., Tow, L. A., Stafford, W., Cary, C. and Cowan, D. A., Bacterial diversity in three different Antarctic cold desert mineral soils. *Microb. Ecol*., 2006, **51**, 413–421.
3. Braun, C., Mustafa, O., Nordt, A., Pfeiffer, S. and Peter, H. U., Environmental monitoring and management proposals for the Fildes region, King George Island, Antarctica. *Polar Res*., 2012, **31**, 18206.
4. Amenábar, M. J., Flores, P. A., Pugin, B., Boehmwald, F. A. and Blamey, J. M., Archaeal diversity from hydrothermal systems of Deception Island, Antarctica. *Polar Biol.*, 2015, **36**, 373–380.
5. Padeiro, A. *et al.*, Trace element contamination and availability in the Fildes Peninsula, King George Island, Antarctica. *Environ. Sci.: Proc. Impacts*, 2016, **18**, 648–657.
6. Yergeau, E., Newsham, K. K., Pearce, D. A. and Kowalchuk, G. A., Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. *Environ. Microbiol*., 2007, **9**(11), 2670–2682.
7. Yergeau, E., Bokhorst, S., Kang, S., Zhou, J., Greer, C. W., Aerts, R. and Kowalchuk, G. A., Shifts in soil microorganisms in response to warming are consistent across a range of Antarctic environments. *ISME J*., 2012, **6**, 692–702.
8. Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. and Glöckner, F. O., Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.*, 2013, **41**(1), e1.
9. Foong, C. P., Wong Vui Ling, C. M. and González, M., Metagenomic analyses of the dominant bacterial community in the Fildes Peninsula, King George Island (South Shetland Islands). *Polar Sci*., 2010, **4**, 263–273.
10. Yergeau, E., Bokhorst, S., Huiskes, A. H. L., Boschker, H. T. S., Aerts, R. and Kowalchuk, G. A., Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. *FEMS Microbiol. Ecol*., 2007, **59**, 436–451.
11. Yu, L., Zhang, W., Liu, L. and Yang, J., Determining Microeukaryotic Plankton Community around Xiamen Island, Southeast China, Using Illumina MiSeq and PCR-DGGE Techniques. *PLoS ONE*, 2015, **10**(5), e0127721.
12. Costello, E. K., Halloy, S. R., Reed, S. C., Sowell, P. and Schmidt, S. K., Fumarole-supported islands of biodiversity within a hyperarid, high-elevation landscape on Socompa Volcano, Puna de Atacama, Andes. *Appl. Environ. Microbiol*., 2009, **75**, 735–747.
13. Zhou, J., Bruns, M. A. and Tiedje, J. M., DNA recovery from soils of diverse composition. *Appl. Environ. Microbiol*., 1996, **62**(2), 316–322.
14. Schloss, P. D. *et al.*, Introducing mothur: an open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol*., 2009, **75**(23), 7537–7541.
15. Huson, D. H., Mitra, S., Weber, N., Ruscheweyh, H.-J. and Schuster, S. C., Integrative analysis of environmental sequences using MEGAN 4. *Genome Res*., 2011, **21**, 1552–1560.
16. Stomeo, F. *et al.*, Abiotic factors influence microbial diversity in permanently cold soil horizons of a maritime-associated Antarctic Dry Valley. *FEMS Microbiol. Ecol.*, 2012, **82**, 326–340.
17. Babalola, O. O., Kirby, B. M., Le Roes-Hill, M., Cook, A. E., Cary, S. C., Burton, S. G. and Cowan, D. A., Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils. *Environ. Microbiol.*, 2009, **11**, 566–576.
18. Bergmann, G. T. *et al.*, The under-recognized dominance of *Verrucomicrobia* in soil bacterial communities. *Soil Biol. Biochem*., 2011, **43**, 1450–1455.
19. Green, J. and Bohannan, B. J. M., Spatial scaling of microbial biodiversity. *Trends Ecol. Evolut*., 2006, **21**, 501–507.
20. Ren, Y., Niu, J., Huang, W., Peng, D., Xiao, Y., Zhang, X., Liang, Y., Liu, X. and Yin, H., Comparison of microbial taxonomic and functional shift pattern along contamination gradient. *BMC Microbiol.*, 2016, **16**, 110.

ACKNOWLEDGEMENTS. This work was supported by the Ministry of Science, Technology and Innovation (MOSTI), Malaysia under the Flagship project FP0712E012. The authors thank personnel at INACH especially José Retamales and Marcelo Leppe for their advice and logistic support for the project.

doi: 10.18520/cs/v115/i9/1701-1705