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Microbial Community of the Arctic Soil from the Glacier Foreland of Midtre Lovénbreen in Svalbard by Metagenome Analysis

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Recent succession of soil microorganisms and vegetation has occurred in the glacier foreland, because of glacier thawing. In this study, whole microbial communities, including bacteria, archaea, and eukaryotes, from the glacier foreland of Midtre Lovénbreen in Svalbard were analyzed by metagenome sequencing, using the Ion Torrent Personal Genome Machine (PGM) platform. Soil samples were collected from two research sites (ML4 and ML7), with different exposure times, from the ice. A total of 2,798,108 and 1,691,859 reads were utilized for microbial community analysis based on the metagenomic sequences of ML4 and ML7, respectively. The relative abundance of microbial communities at the domain level showed a high proportion of bacteria (about 86–87%), whereas archaeal and eukaryotic communities were poorly represented by less than 1%. The remaining 12% of the sequences were found to be unclassified. Predominant bacterial groups included Proteobacteria (40.3% from ML4 and 43.3% from ML7) and Actinobacteria (22.9% and 24.9%). Major groups of Archaea included Euryarchaeota (84.4% and 81.1%), followed by Crenarchaeota (10.6% and 13.1%). In the case of eukaryotes, both ML4 and ML7 samples showed Ascomycota (33.8% and 45.0%) as the major group. These findings suggest that metagenome analysis using the Ion Torrent PGM platform could be suitably applied to analyze whole microbial community structures, providing a basis for assessing the relative importance of predominant groups of bacterial, archaeal, and eukaryotic microbial communities in the Arctic glacier foreland of Midtre Lovénbreen, with high resolution.

Keywords: Arctic, glacier foreland, microbial community, metagenome, Ion Torrent

Introduction

The Arctic environment is characterized by an

extremely cold climate, short growing season, and limited nutrient supply [22]. For the last few decades, the arctic environment has been undergoing large changes because of increase in temperature caused by global warming, increased human activity and a wide-range of transport pollutants [14]. The temperatures in the Arctic have increased twice as much as the global average over the past 100 years, resulting in thawing glacier and exposure of glacier forelands that have long been covered by ice [6, 10, 30]. The forelands exposed after glacial retreat present new habitats for microorganisms that

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then play important roles in biogeochemical cycles, soil development, heterotrophic activities and their associated plant growth [29]. Therefore, the soil microbial communities in the glacier foreland could be key determinants of ecosystem stability and function in the newly exposed region. However, fundamental knowledge regarding microbial communities in the glacier foreland has been insufficient to understand their ecosystems, resulting in the need for studies of microbial diversity and composition in these Arctic environments [9, 29].

Our knowledge regarding microbial diversity has been greatly extended by molecular analysis of certain marker genes including 16S rRNA, *rpoB* (β subunit of RNA polymerase), and *gyrB* (structural gene for the DNA gyrase β subunit) that enable investigation of microbial community structure by providing valuable phylogenetic information in the environmental samples [25, 32]. Since development of the PCR-based 16S rRNA gene cloning technique, various culture-independent approaches targeting effective marker genes or gene regions have been developed and successively applied to assessment of the microbial community structure, including fluorescence in situ hybridization (FISH), rRNA slot-blot hybridization, denaturing gradient gel electrophoresis (DGGE), and terminal restriction fragment length polymorphism (T-RFLP) [16, 24, 26, 27]. However, the aforementioned molecular methods are not adequate to reveal overall microbial communities of certain environments because the techniques are still time-consuming, expensive and unreliable, since analysis of subdominant microbial groups and species provide results with low resolutions [32]. To overcome these limitations, various next-generation sequencing (NGS) platforms have recently been applied to the field of microbial ecology, permitting in-depth sequencing and determination of microbial community dynamics in various types of environments with high taxonomic resolution [20, 38]. These NGS platforms include: 1) 454 sequencing systems as a pyrosequencing-based high-throughput sequencing system, 2) SOLiD and Illumina second-generation sequencing systems based on the oligonucleotide ligation technique, 3) the Ion Torrent Personal Genome Machine (PGM) platform based on semiconductor technology [36]. Since the Ion Torrent PGM was most recently introduced and takes a different approach to sequencing than the other methods, several recent studies have investigated analysis of microbial community dynamics using this platform [34].

Since the end of the Little Ice Age, the glacier has decreased and a new terrestrial ecosystem has appeared in the Midtre Lovénbreen foreland in Svalbard [18]. To date, although vegetation and bacterial succession related to the ecosystem in this area have been studied [10, 15, 21, 25], we still lack detailed information regarding variations in members of the microbial community such as archaea and eukaryotic microorganisms. Therefore, the present study was conducted to investigate microbial communities in Arctic soil samples (ML4 and ML7) of Midtre Lovénbreen in Svalbard by employing the Ion Torrent PGM as NGS platform. The ML4 site was recently exposed from the ice and now contains sparsely distributed plant species. On the contrary, the ML7 site was exposed before the Little Ice Age and contained several plants species growing with a coverage of 40% [13]. Our results will provide suitable application of a scalable and high-throughput Ion Torrent PGM platform for metagenomic analysis of the microbial community structure.

Materials and Methods

Sampling site description

Soil samples (0–5 cm depth) were collected from two regions (ML4 and ML7) on the glacier foreland of Midtre Lovénbreen, Svalbard (78.9ºN, 12.0ºE) [13]. The two groups can be described as follows: the average age of ML4 is 33 years since the glacial retreat, and ML7 is outside the glacier moraine before the Little Ice Age. The physical and chemical properties of soil samples taken from ML4 and ML7 differed from each other. Soil pH of ML4 (pH 8.3) was slightly higher than that of ML7 (pH 7.6). Soil from ML4 was classified as sandy loam, but soil from ML7 was classified as loam. ML7 showed higher soil organic carbon concentration ($2.80 \pm 0.58\%$) than ML4 ($0.44 \pm 0.33\%$). *Saxifraga oppositifolia* was observed first, and its coverage was 10% in ML4, but diverse vascular plants such as *Salix polaris* and *Silene acaulis* covered 40% of the ground in ML7 [13].

Ion Torrent PGM sequencing

Total genomic DNA was extracted using a commercially available DNA extraction kit (FDS-FastDNA

SPIN Kit for soil; MP Biomedicals) [33]. The extracted genomic DNA was stored at -20°C. Library preparation was conducted using the Thermo Scientific™ Museek Library Preparation Kit for Ion Torrent™ with 100 ng of extracted genomic DNAs according to the manufacturer's instructions. Each sample was replicated three times (technical replicates). MuA transposase enzyme catalyzes the fragmentation of DNA and tags the fragments. Platform specific adaptors were added and amplified using a High-Fidelity DNA polymerase. Products of 400 bp to 450 bp were obtained using the E-Gel SizeSelect™ 2% Agarose Gel. The libraries were quantified using an Agilent 2100 Bioanalyzer with High Sensitivity DNA chips following the manufacturer's protocol. Emulsion PCR was performed using an Ion OneTouch 400 Template Kit (Life Technologies, CA, USA) and run on the Ion OneTouch 2 platform. Emulsion PCR products were enriched for templated Ion Sphere™ Particles (ISPs) using the OneTouch ES instrument (Life Technologies) according to the manufacturer's recommendations. Sequencing was performed on the Ion PGM™ System and 318v2 chip using the Ion PGM™ Sequencing 400 Kit (Ion Torrent™, Thermo Fisher) following the manufacturer's recommendations.

Statistical analysis

Taxonomic profiles of the metagenomic reads obtained from Ion Torrent PGM sequencing were assigned using Metagenome Rapid Annotation with the Subsystem Technology (MG-RAST) server (http://metagenomics. nmpdr.org/) [17]. The abundance data were identified via the lowest common ancestor using $1e^{-05}$ as the maximum e-value, a minimum identify of 60%, and a minimum alignment length of 15 as a cutoff. Statistical analysis for distinct taxonomic levels from the MG-RAST server was conducted using the Statistical Analyses of Metagenomic Profiles (STAMP) software v2.0 [23]. To analyze the phylotype diversity, the richness estimators, Chao_1, and Shannon and Simpson diversity indices were calculated using the Species Prediction and Diversity Estimation (SPADE) software [5].

Results and Discussion

Data characteristics

The microbial communities from Arctic soil samples (ML4 and ML7) in the Midtre Lovénbreen, Svalbard were investigated using a metagenome sequencing method. A total of 2,798,108 and 1,691,859 reads with average lengths of 147 and 125 bp were finally obtained after sequence quality control steps (a predicted error rate of one percent, Q20) from ML4 and ML7 samples, respectively. After quality control, 1,768,300 and 793,906 reads of each sample were successively assigned to bacteria, archaea and eukaryotes in the database of the MG-RAST server (Table 1). When microbial communities were analyzed, higher bacterial abundance and diversity were found than that of archaeal or eukaryotic diversity in both sites. At the domain level, the following numbers of bacterial reads 1,526,336 and 692,532 were found to be dominant for ML4 and ML7, respectively. The minor distributions consisted of archaea (11,489 for ML4 and 4,825 for ML7) and eukaryotes (16,990 and 9,257). Although the numbers of reads for each domain in both samples were uneven, comparison among the communities of three domains via diversity indices revealed that the bacterial abundance was higher than that of archaea and eukaryotes, showing that the high operational taxonomic units (OTUs) phylotype richness of bacteria affected the richness estimator, Chao_1 (Table 2). The diversity indices also exhibited consistent patterns with species richness, regardless of sample sites, with the bacterial communities to showing the greatest diversity (Shannon, 6.26 and 6.22; Simpson, 0.00 and 0.00) and archaea showing the lowest diversity (Shannon, 4.31 and 4.28; Simpson, 0.02 and 0.02). This significant difference in diversity between bacterial and other communities was consistent with the results of previously studies [12, 31].

Table 1. Abundance of microbial communities about domain levels in ML4 and ML7 samples.

Reads	Abundance (%)			
	ML4		ML7	
Total	1,768,300	(100.0)	793,906	(100.0)
Bacteria	1,526,336	(86.3)	692,532	(87.2)
Archaea	11,489	(0.6)	4,825	(0.6)
Eukaryotes	16,990	(1.0)	9,257	(1.2)
Viruses	516	(0.0)	65	(0.0)
Other sequences	116	(0.0)	46	(0.0)
Unclassified sequences	1,526	(0.1)	820	(0.1)
Unassigned	211,327	(12.0)	86,361	(10.9)

Table 2. Summary of OUT counts, richness and diversity estimates for the bacteria, archaea and eukaryotes in ML4 and ML7 samples.

Sample		OTU	Chao_1	Shannon	Simpson
Bacteria	ML4	3,388	4,320	6.26	0.00
	ML7	2,853	3,563	6.22	0.00
Archaea	ML4	207	229	4.31	0.02
	ML7	162	188	4.28	0.02
Eukaryotes	ML4	790	1,562	5.15	0.01
	ML7	496	932	5.00	0.01

Bacterial community structure

The bacterial community compositions of the ML4 and ML7 samples were similar and classified into 28 different phyla including *Proteobacteria, Actinobacteria, Planctomycetes, Firmicutes, Verrucomicrobia, Acidobacteria, Cyanobacteria, Bacteroidetes, Chloroflexi*, and other 19 candidates (Fig. 1). The dominant phyla were revealed to be *Proteobacteria* (40.3% and 43.3%) and *Actinobacteria* (22.9% and 24.9%). These two predominant phyla accounted for more than half of the total bacterial sequences. The bacterial community structure exhibited good consistency with the findings of previous studies

showed that *Proteobacteria* and *Actinobacteria* were the dominant bacterial groups in Arctic soil samples [22, 35]. DGGE analysis of an active layer from the Canadian high Arctic also showed that the dominant bands corresponded to *Proteobacteria* and *Actinobacteria* [31]. A few previous studies reported that the metabolically versatile *Actinobacteria*, oligotrophic *Acidobacteria*, and widely distributed *Bacteroidetes* were dominant in Arctic tundra soils [15, 22]. Several genera from the phylum *Actinobacteria* were detected in this study. Specifically, the detection of *Nocardia,* which is known to form mycelia, may be advantageous in oligotrophic environments as its hyphae can absorb water and nutrients. The bacterial community in this study also included *Planctomycetes* (6.4% and 6.0%), *Acidobacteria* (2.8% and 3.8%) and *Bacteroidetes* (4.5% and 3.1%), although these groups were less abundant than others. The abundance of the phylum *Acidobacteria* may reflect the low levels of nutrients present in high Arctic soils and the decrease in soil pH occurring along the chronosequence [11]. The abundance of the phylum *Cyanobacteria* (6.5% and 3.7%) indicates that it is as an essential ecosystem engineer related to the nitrogen fixation process particularly in
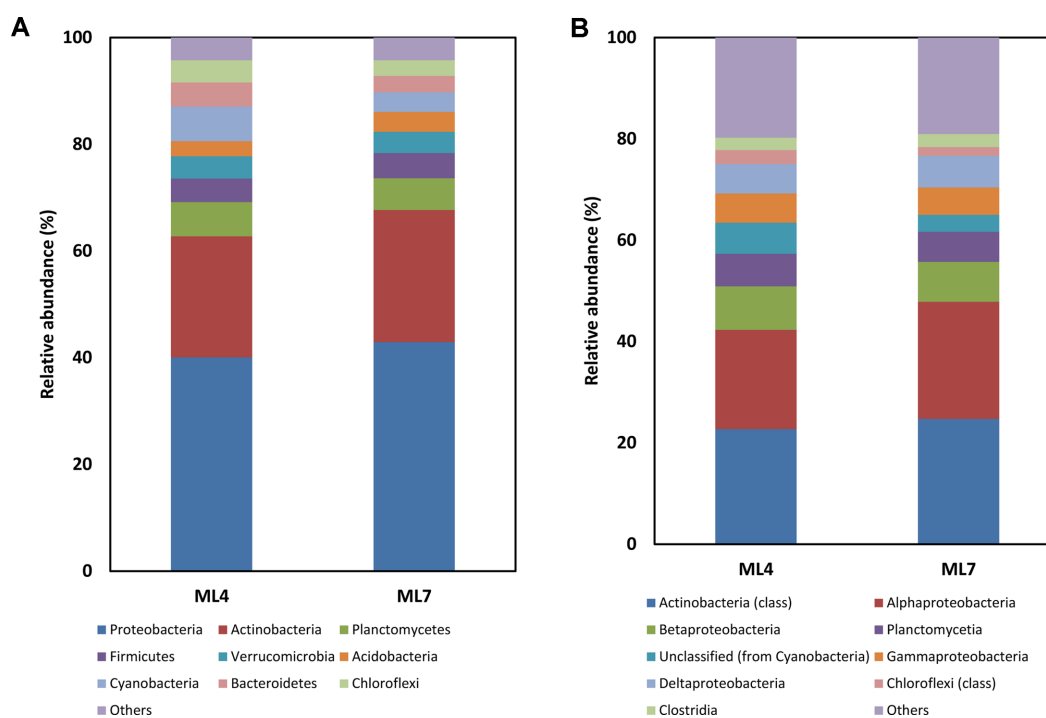

Fig. 1. Relative abundance of phylum (A) and class levels (B) in bacterial community based on OTUs distribution in ML4 and ML7 Arctic soil samples. The combined distribution of minor phyla and classes except those represented as majors was represented to be "Others".

oligotrophic environments such as glacial foreland in Arctic cryoconite communities [7, 8, 27, 28]. While analyzing the methane-oxidizing bacteria within the *Proteobacteria* group, *Beijerinckiaceae* (0.2% and 0.3%) and *Methylocystaceae* (0.1% and 0.2%) were detected as *Alphaproteobacteria* and *Methylococcaceae* (0.2% and 0.2%) as *Gammaproteobacteria* which was in accordance with the results of another investigation of the Midtre Lovénbreen soil of Svalbard, Arctic [13].

Archaeal community structure

In the case of archaeal community structure, ML4 and ML7 samples exhibited a similar composition. When compared to the bacterial community structure, the archaeal community structure was relatively simple, consisting *Euryarchaeota* (84.4% and 81.1%), followed by *Crenarchaeota* (10.6% and 13.1%), an unclassified group (2.6% and 3.1%), *Thaumarchaeota* (1.8% and 2.3%) and minor groups including *Korarchaeota* and *Nanoarchaeota* (Fig. 2). This order of community composition is strongly comparable to that of the subarctic Alaskan tundra based on 454 pyrosequencing [22]. A previous study of the archaeal community structure of

the active layer soil from Resolute in the Canadian High Arctic also supported our results, with *Euryarchaeota* acting as the major archaea group [12]. In addition, the relatively high levels of *Crenarchaeota* and *Thaumarchaeota* were comparable to the results of other studies of the archaeal community structure in the Canadian High Arctic [31]. *Thaumarchaeota* is known to play a major role in geochemical cycles of bioelements including carbon and nitrogen in nature [3, 19].

The major class within the *Euryarchaeota* group was *Methanomicrobia* (34.2% and 34.9%) which is consistent with the results of other studies reporting that the class *Methanomicrobia* is abundant in subarctic Tundra soil [22]. In addition, most archaeal communities found in the active layer and permafrost of the Canadian High Arctic were reportedly composed of *Methanomicrobia* and *Methanobacteria* based on microarray analysis [37]. Among the *Methanomicrobia* class, the abundant genus was revealed to be *Methanosarcina*, followed by *Methanospirillum*, which may be a potentially large source of atmospheric methane [4, 12]. These results combined with the bacterial community structure that consists of methane-oxidizing bacteria strongly imply that the
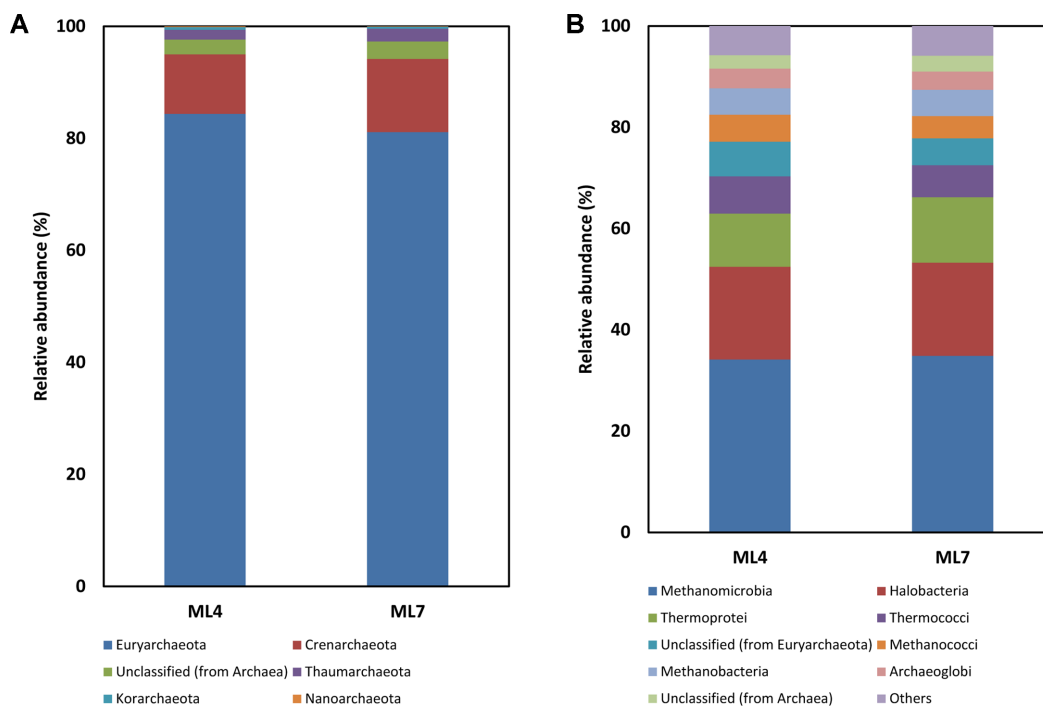

Fig. 2. Relative abundance of phylum (A) and class levels (B) in archaeal community based on OTUs distribution in ML4 and ML7 Arctic soil samples. The combined distribution of minor phyla and classes except those represented as majors was represented to be "Others".

simultaneous production and consumption of methane is one of the key components of the carbon cycle in the Midtre Lovénbreen foreland in Svalbard.

Eukaryotic community structure

The eukaryotic community in ML4 and ML7 was found to be diverse, predominantly composed of *Ascomycota* (33.8% and 50.0%), *Streptophyta* (19.3% and 14.6%) and *Chordata* (14.0% and 12.9%) (Fig. 3). Although most studies have focused on analysis of bacterial and archaeal communities in Arctic environments, the fungal community composition in Svalbard was recently studied by cloning and sequencing the internal transcribed spacer (ITS) fragments. In that study, the major groups of Arctic *Dryas octopetala* root-associated fungal community were *Basidiomycota* (68.8%) and *Ascomycota* (30.7%) [1]. Another similar study showed that the Arctic *Bistorta vivipara* root-associated fungal community in Svalbard had the same major groups based on the 454 pyrosequencing approach [2]. Although *Basidiomycota* was a relatively minor group (2.8% and 3.3%) in our study, the major abundance of *Ascomycota* was comparable to that of previous studies. This incongruence

might be due to the different methods of microbial community analysis, of which the sequence depth in the approaches using the sequencing of ITS fragments and pyrosequencing are insufficient to fully recover the diversity of microbial communities, resulting in the recovered taxonomic profile being incomplete. In another study, *Saxifraga oppositifolia* was regularly observed in the ML4 region, whereas vascular plants such as *Salix polaris* and *Silene acaulis* were diversely distributed in the ML7 region [13]. In addition, the Arctic *Dryas octopetala* was not observed in either region due to its growth condition as dry environments. *Basidiomycota* is represented by fungal biomass in the form of extraradical mycelium and thick ectomycorrhizal (ECM) mantles, whereas *Ascomycota* was found to have a sparse amount of external mycelium, thin mantles and endophytes [1]. A previous study of the phylogenetic relationship between arctic endophytes and plants living in the arctic Tundra in Nunavut, Canada showed that the *Dothideomycetes*, *Sordariomycetes* and *Leotiomycetes* support our findings, with the major class of *Ascomycota* being *Sordariomycetes* (12.3% and 12.9%), *Leotiomycetes* (3.2% and 3.0%), and *Dothideomycetes* (2.5% and 3.9%).
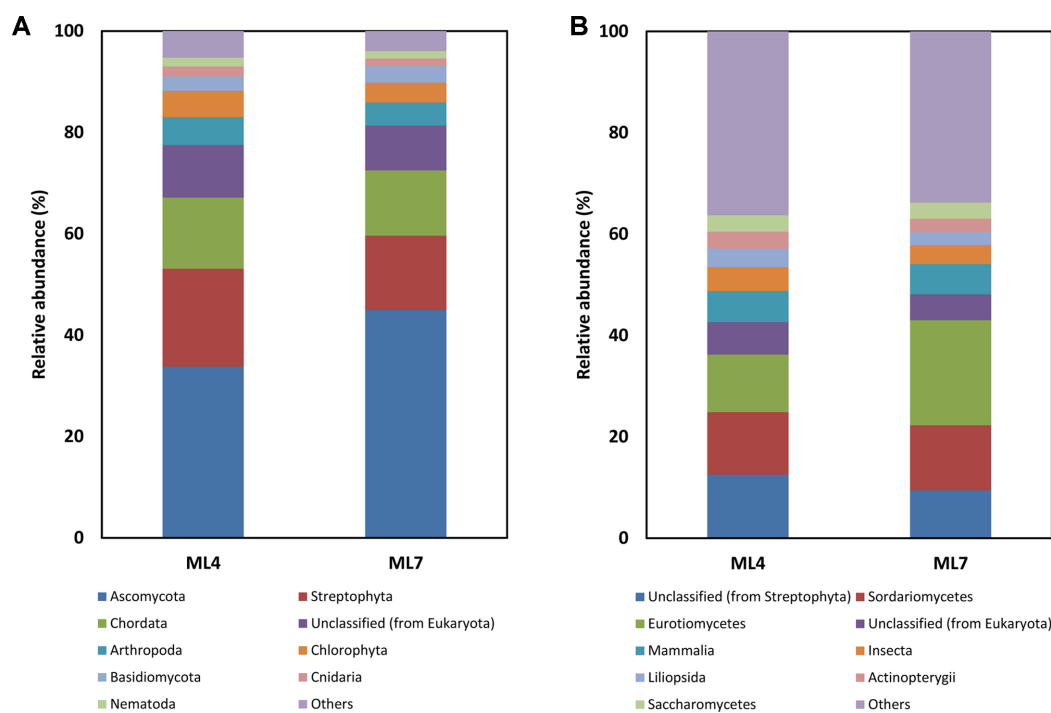

Fig. 3. Relative abundance of phylum (A) and class levels (B) in eukaryotic community based on OTUs distribution in ML4 and ML7 Arctic soil samples. The combined distribution of minor phyla and classes except those represented as majors was represented to be "Others".

In summary, the bacterial, archaeal and eukaryotic communities in Arctic soil from the glacier foreland of Midtre Lovénbreen in Svalbard were examined with a high resolution by using the scalable and rapid NGS technologies, in particular, the Ion Torrent PGM platform. Analysis of soil microorganisms other than bacteria has been limited in this region due to the low mass of microorganisms; however, this NGS-based metagenomic approach could address this limitation, enabling analysis of the whole microbial community structures including bacteria, archaea and even eukaryotes more rapidly, than previously employed molecular methods such as PCR-based 16S rRNA gene cloning, DGGE and pyrosequencing. There have been many studies of the microbial community structures in Svalbard Arctic regions and it is difficult to characterize and compare microbial communities in Arctic soil samples. However, the results of the present study are concordant with those of other previous studies of microbial ecosystems in Arctic region.

Acknowledgments

References

1. Bjorbækmo MFM, Carlsen T, Brysting A, Vrålstad T, Høiland K, Ugland KI, et al. 2010. High diversity of root associated fungi in both alpine and arctic *Dryas octopetala*. *BMC Plant Biol.* **10**: 244.

2. Blaalid R, Davey ML, Kauserud H, Carlsen T, Halvorsen R, Høiland K, Eidesen PB. 2014. Arctic root-associated fungal community composition reflects environmental filtering. *Mol. Ecol.* **23**: 649–659.

3. Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P. 2008. Mesophilic *Crenarchaeota*: proposal for a third archael phylum, the *Thaumarchaeota*. *Nat. Rev. Microbiol.* **6**: 245–252.

4. Cadillo-Quiroz H, Yahiro E, Yavitt JB, Zinder SH. 2008. Characterization of the archaeal community in a minerotrophic fen and terminal restriction fragment length polymorphism-directed isolation of a novel hydrogenotrophic methanogen. *Appl. Environ. Microbiol.* **74**: 2059–2068.

5. Chao A, Shen TJ. 2003. Program SPADE (Species Prediction and Diversity Estimation) Program and user's guide available at http://chao.stat.nthu.edu.tw.

6. Coulson SJ, Hodkinson ID, Webb NR. 2003. Aerial dispersal of invertebrates over a high-Arctic glacier foreland: Midtre Lovénbreen, Svalbard. *Polar Biol.* **26**: 530–537.

7. Edwards A, Mur LAJ, Girdwood SE, Anesio AM, Stibal M, Rassmer AME, et al. 2014. Coupled cryoconite ecosystem structure-function relationships are revealed by comparing bacterial communities in alpine and Arctic glaciers. *FEMS Microbiol. Ecol.* **89**: 222–237.

8. Evonne PYT, Warwick FV, Proulx D, Noüe PLJ. 1997. Polar cyanobacteria versus green algae for tertiary waste-water treatment in cool climates. *J. Appl. Phycol.* **9**: 371–381.

9. Green J, Bohannan JM. 2006. Spatial scaling of microbial biodiversity. *Trends Ecol. Evol.* **21**: 501–507.

10. Hinzman LD, Bettez ND, Bolton WR, Chapin FS, Dyurgerove MB, Fastie CL, et al. 2005. Evidence and implications of recent climate change in northern Alaska and other arctic regions. *Clim. Change* **72**: 251–298.

11. Hodkinson ID, Coulson SJ, Webb NR. 2003. Community assembly along proglacial chronosequences in the high Arctic: vegetation and soil development in north-west Svalbard. *J. Ecol.* **91**: 651–663.

12. Kim OS, Kim HM, Lee HK, Lee YK. 2014. Microbial community structure of the active layer soil from Resolute, Canadian high Arctic. *J. Climate Change Res.* **5**: 249–256.

13. Kwon HY, Jung JY, Kim OS, Laffly D, Lim HS, Lee YK. 2015. Soil development and bacterial community shifts along the chronosequence of the Midtre Lovénbreen glacier foreland in Svalbard. *J. Ecol. Environ.* **38**: 461–476.

14. Larose C, Prestat E, Cecillon S, Berger S, Malandain C, Lyon D, et al. 2013. Interactions between snow chemistry, mercury inputs and microbial population dynamics in an arctic snowpack. *PLoS One* **8**: e79972.

15. Lee SH, Jang I, Chae N, Choi T, Kang H. 2013. Organic layer serves as a hotspot of microbial activity and abundance in Arctic tundra soils. *Microb. Ecol.* **65**: 405–414.

16. Llobet-Brossa E, Rossello-Mora R, Amann R. 1998. Microbial community composition of Wadden sea sediments as revealed by fluorescence in situ hybridization. *Appl. Environ. Microbiol.* **64**: 2691–2696.

17. Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, et al. 2008. The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* **9**: 386.

18. Moreau M, Mercier D, Laffly D, Rousse E. 2008. Impacts of recent paraglacial dynamics on plant colonization: A case study on Midtre Lovénbreen foreland, Spitsbergen (79°N). *Geomorphology* **95**: 48–60.

19. Muller F, Brissac T, Le Bris N, Felbeck H, Gros O. 2010. First description of giant *Archaea* (*Thaumarchaeota*) associated with putative bacterial ectosymbionts in a sulfidic marine habitat. *Environ. Microbiol.* **12**: 2371–2383.

20. Nam YD, Kim HJ, Seo JG, Kang SW, Bae JW. 2013. Impact of pelvic radiotheraphy on gut microbiota of gynecological cancer patients revealed by massive pyrosequencing. *PLoS One* **8**:

e82659.

21. Nilsen L, Brossard T, Joly D. 1999. Mapping plant communities in a local Arctic landscape applying a scanned infrared aerial photograph in a geographical information system. *Int. J. Remote Sens.* **20**: 463–480.

22. Park HJ, Chae N, Sul WJ, Lee BY, Lee YK, Kim D. 2015. Temporal changes in soil bacterial diversity and humic substances degradation in subarctic tundra soil. *Microb. Ecol.* **69**: 668–675.

23. Parks DH, Beiko RG. 2010. Identifying biologically relevant differences between metagenomics communities. *Bioinformatics* **26**: 715–721.

24. Pereira e Silva MC, Dias ACF, van Elsas JD, Salles JF. 2012. Spatial and temporal variation of archaeal, bacterial and fungal communities in agricultural soils. *PLoS One* **7**: e51554.

25. Ravenschlag K, Sahm K, Amann R. 2001. Quantitative molecular analysis of the microbial community in marine Arctic sediments (Svalbard). *Appl. Environ. Microbiol.* **67**: 387–395.

26. Sahm K, MacGregor BJ, Jørgensen BB, Stahl DA. 1999. Sulfate reduction and vertical distribution of sulfate-reducing bacteria quantified by rRNA slot-blot hybridization in a coastal marine sediment. *Environ. Microbiol.* **1**: 65–74.

27. Schmidt SK, Reed SC, Nemergut DR, Grandy AS, Cleveland CC, Weintraub MN, *et al*. 2008. The earliest stages of ecosystem succession in high-elevation (5000 metres above sea level), recently deglaciated soils. *Proc. Biol. Sci.* **275**: 2793–2802.

28. Schulz S, Brankatschk R, Dümig A, Kögel-Knabner I, Schloter M, Zeyer J. 2013. The role of microorganisms at different stages of ecosystem development for soil formation. *Biogeosciences* **10**: 3983–3996.

29. Schütte UME, Abdo Z, Foster J, Ravel J, Bunge J, Solheim B, *et al*. 2010. Bacterial diversity in a glacier foreland of the high Arctic. *Mol. Ecol.* **19**: 54–66.

30. Serreze MC, Walsh JE, Chapin FS, Osterkamp T, Dyurgerov M, Romanovsky V, *et al*. 2000. Observational evidence of recent change in the northern high-latitude environment. *Clim. Change.* **46**: 159–207.

31. Steven B, Pollard WH, Greer CW, Whyte LG. 2008. Microbial diversity and activity through a permafrost/ground ice core profile from the Canadian high Arctic. *Environ. Microbiol.* **10**: 3388–3403.

32. Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M. 2014. Development of a prokaryotic universal primer for simultaneous analysis of *Bacteria* and *Archaea* using next-generation sequencing. *PLoS One* **9**: e105592.

33. Vishnivetskaya TA, Layton AC, Lau MC, Chauhan A, Cheng KR, Meyers AJ, *et al*. 2014. Commercial DNA extraction kits impact observed microbial community composition in permafrost samples. *FEMS Microbiol. Ecol.* **87**: 217–230.

34. Whiteley AS, Jenkins S, Waite I, Kresoje N, Payne H, Mullan B, *et al*. 2012. Microbial 16S rRNA Ion Tag and community metagenome sequencing using the Ion Torrent (PGM) Platform. *J. Microbiol. Methods* **91**: 80–88.

35. Wilhelm RC, Niederberger TD, Greer C, Whyte LG. 2011. Microbial diversity of active layer and permafrost in an acidic wetland from the Canadian High Arctic. *Can. J. Microbiol.* **57**: 303–315.

36. Yang Y, Xie B, Yan J. 2014. Application of next-generation sequencing technology in forensic science. *Genomics Proteomics Bioinformatics* **12**: 190–197.

37. Yergeau E, Hogues H, Whyte LG, Greer CW. 2010. The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. *ISME J.* **4**: 1206–1214.

38. Yergeau E, Lawrence JR, Sanschagrin S, Waiser MJ, Korber DR, Greer CW. 2012. Next-generation sequencing of microbial communities in the Athabasca River and its tributaries in relation to oil sands mining activities. *Appl. Environ. Microbiol.* **78**: 7626–7637.

국문초록

북극 스발바르 군도 중앙로벤 빙하 해안 지역의 토양 시료 내 메타지놈 기반 미생물 군집 분석
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최근 빙하의 융해로 인해 빙하 해안 지역에 다양한 토양 미생물과 초목들이 드러나고 있다. 본 연구에서는 북극 스발바르 군도 중앙로벤 빙하 해안 지역으로부터 Ion Torrent Personal Genome Machine(PGM)을 활용한 메타지놈 분석을 통해 세균(bacteria), 고균(archaea), 및 진핵생물(eukaryotes)를 포함하는 다양한 미생물 군집을 분석하였다. 연구에 사용된 토양시료는 빙하 후퇴에 따른 토양의 노출 시기에 따라 2개 지역(ML4 및 ML7)으로부터 수집하였다. ML4 및 ML7 시료의 메타지놈 염기서열을 기반으로 총 2,798,108 및 1,691,859 reads가 각각 미생물 군집 분석에 활용되었다. Domain (계) 수준에서 미생물 군집의 상대 빈도를 분석한 결과 2개 시료 모두 세균(86–87%)이 높은 반면 고균과 진핵생물은 1% 미만으로 존재하는 것으로 나타났다. 또한 약 12%의 염기서열은 기존에 분류되지 않은(unclassified) 서열로 분석되었다. 세균의 경우 *Proteobacteria*(40.3% for ML4 and 43.3% for ML7)와 *Actinobacteria*(22.9% and 24.9%)가 우점하는 것으로 분석되었다. 고균의 경우에는 *Euryarchaeota*(84.4% and 81.1%) 및 *Crenarchaeota*(10.6% and 13.1%), 그리고 진핵생물의 경우에는 *Ascomycota*(33.8% and 45.0%)가 우점하는 것으로 분석되었다. 본 연구를 통해 Ion Torrent PGM 플랫폼을 활용한 메타지놈 분석이 북극의 중앙로벤 빙하 해안 지역의 전체 미생물 군집 구조를 파악하는데 충분히 적용될 수 있을 것으로 사료된다.