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# RESEARCH ARTICLE

# Comparing rock-inhabiting microbial communities in different rock types from a high arctic polar desert

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**One sentence summary:** Rocks in high Arctic polar desert act as reservoirs of diverse bacteria and fungi, and the dominant microorganisms were closely affiliated to those found in other cold deserts.

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## ABSTRACT

Although rocks are habitable places for microbes in extreme environments, microbial diversity in these lithic environments is still poorly understood. The diversity and abundance of rock-inhabiting microbial communities in different types of rock in Svalbard, Norwegian High Arctic were examined using NGS sequencing of bacterial 16S rRNA genes and fungal 28S rRNA genes. Compositions of both bacterial and fungal communities varied across different rock types: sandstone, limestone, basalt, granite and travertine. Bacterial communities were dominated by Actinobacteria, Proteobacteria, Chloroflexi, Bacteroidetes and Acidobacteria. Fungal communities consisted of Eurotiomycetes, Lecanoromycetes, Dothideomycetes and Leotiomycetes. Both bacterial and fungal communities were considerably correlated with the geochemical characteristics of rocks. Bacterial communities were considerably correlated with the rock elements such as Mg and Ca. Fungal communities were considerably correlated with the rock elements such as Mg and Ca. Fungal communities were considerably area were closely affiliated to those found in other cold regions such as Alpine area, Arctic and Antarctica, suggesting that environmental constraints such as cold temperature may lead to convergence in microbial community composition. These results confirm that rocks in cold environments act as reservoirs of diverse bacteria and fungi, which may improve our understanding of lithic microbial ecology in the cold desert.

Keywords: Arctic; extreme environment; rock; lithic microbial community; bacteria; fungi

#### **INTRODUCTION**

Polar regions have extremely low temperatures, dry atmosphere, strong ultra violet radiation, low nutrient availability and long periods without sunlight. Microorganisms living in these environments may find a sheltered habitat in rocks to protect themselves from environmental stresses (Walker and Pace 2007b). Thus, rock-inhabiting microbes are a major focus of many investigations of life in harsh environments or studies with astrobiological implications (Friedmann 1980; Friedmann et al. 1993; Parnell et al. 2004; De los Rios et al. 2005; Wierzchos, Ascaso and

McKay 2006; Wierzchos et al. 2013). Such studies require identification of microbial composition to understand how characteristics of rock and habitat features affect microbial communities.

According to previous studies, rock microbial colonization is influenced by physical and chemical properties of rock substrates, such as pore structure, mineral composition and permeability, as well as environmental factors such as climatic exposure, nutrient sources and water availability (Friedmann *et al.* 1993; Warscheid and Braams 2000; Omelon, Pollard and Ferris 2006a). Many studies on lithic microbes have been conducted in various lithologies and localities. Regardless of rock type,

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lithic microbial colonization has been recorded in various sedimentary rocks such as sandstone (Friedmann 1980; Friedmann et al. 1993; Omelon, Pollard and Ferris 2006a), limestone (Ferris and Lowson 1997; Wong et al. 2010), gypsum (Hughes and Lawley 2003; Boison et al. 2004; Dong et al. 2007), dolomite (Sigler, Bachofen and Zeyer 2003), carbonate (Hoppert et al. 2004) and halite (Wierzchos, Ascaso and McKay 2006). In addition, microbial colonization has been detected on igneous rocks such as granite (De los Rios et al. 2005; Gleeson et al. 2005), ignimbrite (Wierzchos et al. 2013) and basalt (Izawa et al. 2010). Furthermore, a few investigations have focused on microbial communities associated with terrestrial volcanic deposits (King 2003; Dunfield and King 2004; Gomez-Alvarez, King and Nusslein 2007; Herrera et al. 2008; Costello et al. 2009). These studies have shown how differences in rock properties affected the microbial composition.

Geographically, microbial colonization in rocks has been observed in a variety of extreme terrestrial ecosystems, including rocky outcrops in high-altitude regions (Ferris and Lowson 1997; Wong et al. 2010), semi-arid areas (Sigler, Bachofen and Zeyer 2003), hyper-arid deserts (Wierzchos, Ascaso and McKay 2006; Dong et al. 2007; Wierzchos et al. 2013) and cold deserts such as in the Arctic and Antarctica (Hughes and Lawley 2003; De los Rios et al. 2005). In these environments, microorganisms colonize rock substrates inside and underside to adapt to diverse stresses such as aridness and ultra violet radiation (Hughes and Lawley 2003; De los Rios et al. 2005; Wierzchos, Ascaso and McKay 2006; Dong et al. 2007; Wierzchos et al. 2013). Notably, results from morphological and molecular analyses revealed that closely affiliated microbial lineages are often shared across rocks in different geographical regions, implying that the taxonomic commonality may result from the selection of geographically dispersed lineages under similar environmental conditions of lithic habitats (Walker and Pace 2007a).

As described above, although rock-colonizing microbes are widespread in a variety of geographical regions, very little information is available about what microbial communities are inhabiting lithic habitat in the Norwegian high Arctic polar desert (Starke *et al.* 2013).

Svalbard is an archipelago in Arctic Ocean, located the north-western margin of the Barents Sea Shelf. The area has a very diverse and long geologic history. The geological record of Svalbard from Archaean to Recent is divided into three parts: the basement, unaltered sedimentary rocks and superficial (unconsolidated) deposits (Dallmann 2007). Spitsbergen, which includes our study area, is the largest island of the Svalbard. The basement of southern Spitsbergen consists of phyllite, quartzite, limestone, dolostone and conglomerate, and minor amounts of volcanic rocks. On the other hand, the basement of north (north-east) Spitsbergen is composed of gneiss, migmatite and granite, commonly with inclusions of schist, marble and quartzite. The most common rock-forming minerals are quartz, feldspar, calcite, dolomite, biotite, muscovite, amphibole, pyroxene, garnet, chlorite and olivine (Hjelle 1993). Thus, this area is a suitable environment for studying various rock-inhabiting microorganisms.

In this study, we examined the community structure of bacteria and fungi inhabiting different types of rock collected from four sites in Svalbard. The main objectives of this study were (1) to investigate the major members of the bacterial and fungal communities colonizing the lithic environments in this cold and dry region and (2) to investigate whether microbes that are the same as or closely related to those in this environment can also be found in other environments by searching databases for taxa from other studies.

## MATERIALS AND METHODS

# Geomorphologic and geologic characterization of study sites

Sampling sites were located in areas around Ny-Ålesund (78°55′28″N, 11°55′42″E) and Bockfjorden (79°28′08″N, 13°19′43″E) in Svalbard: Smithelva, Stupbekken, Troll spring and Halvdanpiggen (Fig. 1). Rock samples were collected from grounds bare of mosses and lichens. Sampling site 1 (Smithelva) was located ca. 1 km SE of Ny-Ålesund. Chert, cherty limestone and sandstone of the Permian and Triassic ages were distributed on several strips of chert beds, and pebble- to boulder-sized rubble from the outcrop covered most of the land area. The rocks were predominantly brown in color on weathered surfaces of the outcrop (Fig. 2a). Discontinuous patches of light brown surface crust appeared on these outcrops, providing evidence of significant exfoliation of the rock surface.

Sampling site 2 (Stupbekken) was located in a valley ca. 6 km NW of Ny-Ålesund. This area is situated 500 m from the coast and at an altitude of 168 m (upper site) and 63 m (lower site). Bedrock of the Stupbekken was composed predominantly of dolomitic limestone of the Carboniferous age and formed a steep cliff (ca. 10 m high) facing NE near the lower site. The apron that has developed along the base of the cliff, and the top of the cliff is mostly covered by dolomitic limestone rubble of various shapes and sizes, from boulders to small pebbles (Fig. 2b and c). Owing to continuous erosion and mobilization of rubble, the dolomitic limestone of the area was exposed to the atmosphere without vegetation.

Sampling site 3 (Troll spring) is linked to the Cenozoic Bockfjorden Volcanic Complex, which was located along a major N-S trending fault-zone separating the Devonian red sandstones from the Proterozoic basement rocks (Fig. 2d and e). The spring water of the volcanic system has precipitated Ca carbonates, producing meter-scale terraces. Compared to the wet environment around the source, the lower terraces were partially or completely dry. As a result, from the source pool to the terraces, environmental conditions changed from wet to dry, warm to cold (Starke *et al.* 2013).

Sampling site 4 (Halvdanpiggen) was a ruin of Cenozoic volcanic pipe (Fig. 2f). The volcanic rocks intruded into the Devonian red sandstones. Halvdanpiggen is a volcanic neck, measuring 200 m across, consisting of a pyroclastic breccia of basaltic blocks and 60%–70% angular high-pressure xenoliths. The central section of Halvdanpiggen was intruded by aphanitic nonvesicular basalts weathering with a fanning columnar jointing. The estimated terrain elevation was 834 m above sea level (Skjelkvale *et al.* 1989).

#### **Rock sampling**

A total of 23 rock samples were collected from the four study sites in Svalbard, Norway, in August 2014 (see Table S1, Supporting Information). In each site, two or more rocks per rock type were collected. Three different parts of each rock or outcrop pooled into a sterile plastic bag to make a composite rock sample. The rock samples were transported to the laboratory in an icebox at  $\leq 4^{\circ}$ C. To obtain all the microorganisms living in the rock, we crushed whole rock using Mixer Mill (Retsch, Germany)



Figure 1. Map of study area in Svalbard. Rock sampling sites are indicated by red circles. The figure was prepared based on the map of Toposvalbard made by the Norwegian Polar Institute.



Figure 2. Landscape of sampling sites. (a) Smithelva. (b) Stupbekken-upper. (c) Stupbekken-lower. (d) Troll spring-upper. (e) Troll spring-lower. (f) Halvdanpiggen.

without differentiating surface and interior parts. The powdered rocks were used to extract gDNA.

#### Scanning electron microscopy analysis

In order to characterize the fine details of the endolithic colonies in the rocks, we employed Scanning electron microscopy (SEM). Rocks were fractured to expose the interior that included the region encompassing endolithic microorganisms. Rock fragments were fixed in 2.5% glutaraldehyde for 8 h, air-dried overnight and gold sputter-coated for 30 s (Cressington 108 Auto; Cressington Scientific Instruments Ltd, UK) prior to visual examination with a JSM-6610LV machine (JEOL, USA).

#### X-ray fluorescence spectrometry analysis

Whole-rock major element compositions were obtained using a wavelength-dispersive X-ray fluorescence spectrometry (XRF) spectrometer (Panalytical, the Netherlands). XRF analysis was carried out on glass discs prepared by fusing one part of a finely powdered sample with five parts of LT100 flux (100% Li tetraborate; XRF Scientific, Australia). Three discs were made per each sample, and each disc was measured three times.

#### Total rock DNA extraction, polymerase chain reaction amplification and NGS sequencing

Total genomic DNA was extracted from 3 g of freeze-dried rock powder, using the FastDNA SPIN kit (MP Biomedicals, Illkirch, France) according to the manufacturer's instructions. The extracted DNA was stored at  $-20^{\circ}$ C until further analysis. Extractions were carried out in triplicate on all samples and the resulting nucleic acids pooled for better representation. The extracted DNA was amplified using primers targeting the V3-V4 region of the bacterial 16S rRNA and the D1-D2 region of the fungal 28S rRNA (Rehner and Samuels 1994; Klindworth et al. 2013) (see Table S2, Supporting Information). The polymerase chain reaction (PCR) reaction mixture (50  $\mu$ L) contained 25  $\mu$ L of master mix (DreamTaq Green PCR Master Mix; Thermo scientific, USA), 1  $\mu$ L of the forward and reverse primers (10 pmol of each primer), 1  $\mu$ L of template DNA and 23  $\mu$ L of deionized distilled water (DDW). The PCR conditions were as follows: initial denaturation for 3 min at  $95^{\circ}C$ , followed by 25 cycles of denaturation (30 s, 95°C), annealing (30 s, 55°C), extension (30 s, 72°C) and final extension at 72°C for 5 min. Amplicons from three reactions were pooled together for sequencing. We tried amplification in all the samples, but bacterial 16S rRNA gene was amplified in only 10 samples. Fungal 28S rRNA gene was amplified in only six samples. For bacteria, paired-end sequencing was performed at ChunLab Inc. (Seoul, Republic of Korea) using 2  $\times$  300 bp MiSeq (Illumina) runs according to the manufacturer's instructions. Paired-end reads were assembled using PANDAseq (Masella et al. 2012), and assembled reads were processed following the Mothur MiSeq SOP (Kozich et al. 2013). For fungi, pyrosequencing was performed by Macrogen Inc. (Seoul, Republic of Korea) using the 454 GS FLX Titanium Sequencing system (Roche, NJ, USA) according to the manufacturer's instructions. Raw reads were initially processed using PyroTrimmer (Oh et al. 2012), followed by quality-trimming, denoising and chimera detection by Mothur 454 SOP (Schloss, Gevers and Westcott 2011). Quality-filtered sequences were taxonomically assigned against the EzTaxon-e database for bacteria (Kim et al. 2012) and the ribosomal database project (RDP) fungal large subunit reference data for fungi, using the naïve Bayesian classifier implemented in Mothur. Operational taxonomic units (OTUs) were defined at a 97% sequence similarity level for both bacteria and fungi. Raw sequence data were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession number SRP102559.

#### Statistical analysis

Diversity indices were generated based on OTUs, using Mothur (Schloss *et al.* 2009). Bray-Curtis dissimilarities between samples were calculated using the hellinger-transformed OTU matrix and untransformed phylum abundance matrix. A Mantel test was performed to determine which mineral properties of rock were significantly correlated with the lithic microbial communities. Differentially abundant taxa between carbonate rock and non-carbonate rock were identified using Metastats (White, Nagarajan and Pop 2009). All statistical analyses were performed using R version 3.1.2 (www.r-project.org) and PRIMER-E v6.1.11 (Clarke and Gorley 2006).

#### RESULTS

#### Visualization of rock-inhabiting microbes by microscopy

We collected different types of rocks such as sandstone, limestone, granite, basalt and travertine from Svalbard (Fig. 3a–f). SEM of the rock samples clearly showed microbial structures (Fig. 3g–l). Both rod-shaped bacteria and cocci were clearly visible in rock samples basalt (Fig. 3g), granite (Fig. 3h) and travertine (Fig. 3i). Filamentous structure with branches was also observed, which were similar with the hyphae of fungi in sandstone (Fig. 3j) and limestone (Fig. 3k and l).

# Molecular characterization of rock-inhabiting microbial communities

DNA sequencing was performed to examine the community structure of rock-inhabiting bacteria and fungi, and the sequencing results are summarized in Table S3 (Supporting Information). For bacteria, 29004 high quality sequence reads in total were obtained from 10 samples. The number of sequence reads per sample ranged from 2687 to 3187, with a mean of 2900 reads per sample (see Fig. S3, Supporting Information). Although the number of reads is low, Good's coverage of overall sequence was more than 94%, so it is not insufficient to explain majority of bacterial phylotypes in the samples (see Table S3, Supporting Information). Bacterial alpha-diversity differed considerably across different rock types. The highest level of bacterial diversity was observed in a limestone (DS3), whereas the lowest level was found in a basalt stone (L23).

A total of 34 bacterial phyla were identified, of which nine phyla were present in all types of rock isolated in this study. Actinobacteria was the most abundant phylum (38.6% on average) across the entire sample set (Fig. 4a), followed by Proteobacteria (21.8%), Cyanobacteria (12.6%), Chloroflexi (6.3%), Bacteroidetes (5.5%) and Acidobacteria (4.4%). Other phyla such as Deinococcus-Thermus (3.2%), Verrucomicrobia (1.6%) and Planctomycetes (1.4%) were also present consistently across all samples, but in lower abundance. The greatest range of abundance variation was found in Actinobacteria (11.0%–83.8%) and to a lesser extent in Cyanobacteria (0%–36.7%) and Proteobacteria (2.2%–35.9%).

The relative abundance of bacterial phyla varied considerably between rock types (Fig. 4a). The basalt showed a unique microbial structure, with the highest proportion of *Actinobacteria* 



Figure 3. Fractured rocks and SEM images of endolithic microorganisms. (a-f) Pictures showing fractured rock samples. SEM images showing bacterial aggregates on fractured basalt (g), granite (h) and travertine (i). SEM images showing fungal hyphae on fractured sandstone (j) and limestone (k and l).

(> 80%) and a very low proportion of Proteobacteria (< 2.3%). Limestones and travertines contained a relatively high proportion of Cyanobacteria (> 12% and > 22%, respectively). Deinococcus-Thermus was mostly found in travertine from Troll spring (> 5.5%). At the family level, the bacterial composition also differed along the rock types (see Fig. S1, Supporting Information). For example, among the actinobacterial family Rubrobacteraceae was highly abundant in basalt, and Micrococcaeae was present relatively at higher abundance in both basalt and granite. Those two families being considerably less abundant in other rock types. An unknown Rhodospirillales family (Acetobacteraceae.f) of Alphaproteobacteria was relatively abundant in two local sites (Smithelva and Stupbekken) of the Brøgger Peninsula, whereas an unknown Caulobacterales family (Caulobacteraceae.f) was considerably abundant only in a travertine sample, L15. In Bacteroidetes, an unknown Sphingobacteriales family (Chitinophagaceae\_f) occurred consistently across all samples at disproportionate rates, and an unknown Cytophagales family (Cyclobacteriaceae\_f) was present in the majority of travertine samples but virtually absent in other sites.

The quantitative mineral composition of all rock samples was determined by XRF (see Table S4, Supporting Information). The overall bacterial community structure was considerable related to the rock element composition (Mantel test, P < 0.05), and significant correlations were also observed in individual phyla such as Actinobacteria, Bacteroidetes, Cyanobacteria, and Proteobacteria (see Table S5, Supporting Information). Actinobacteria, Bacteroidetes, Cyanobacteria, Bacteroidetes, Cyanobacteria, and Proteobacteria exhibited a significant positive relationship with Si, Al, Fe, Mg, P, K and Ti (P < 0.05 in all cases), while Chloroflexi showed a significant correlation with Si, P and Ca (r = 0.35, 0.45 and 0.33, respectively). Cyanobacteria was strongly correlated with Fe, Mg, P and Ti (r = 0.65, 0.62, 0.63 and 0.68, respectively). We further investigated the effects



**(b)** 



Figure 4. Clustering pattern of rock samples based on bacterial phylum composition (a) and fungal class composition (b). Circles and squares represent locality of rock sampling and lithology of rocks, respectively. The dendrogram at the top was generated using the Bray-Curtis dissimilarity of bacterial phylum abundance between samples (a) and fungal class abundance between samples (b).

of substrate mineralogy on bacterial community structure using Metastats. Various genera were preferentially abundant in carbonate rocks (see Table S6, Supporting Information). For example, an unknown cyanobacterial genus (ALVU\_g) (5.1%), *Truepera* (4.8%), an unknown *Chloroflexi* genus (GQ396871\_g) (1.0%) and several genera were abundant in carbonate rocks. These results are consistent with the Mantel test result that showed a significant correlation between the Ca and bacterial phyla.

For fungi, we obtained a total of 24282 high-quality 28S rRNA sequence reads from six samples. The number of sequence reads varied between samples, ranging from 378 to 8000 with a mean of 4047 reads per sample (see Table S3, Supporting Information). The highest level of fungal diversity was observed in a sandstone sample (DS1).

Four fungal phyla were present across all samples. Ascomycota was the most abundant phylum (96.0% on average) across the entire sample set, followed by Basidiomycota (0.1%) and Chytridiomycota (0.05%), and unclassified phyla were present at 1.2%. At the class level, the rock fungal communities (Fig. 4b) were dominated by four fungal classes: Eurotiomycetes (30.0% on average), Lecanoromycetes (23.0%), Leotiomycetes (12.1%), and Dothideomycetes (8.1%). Other classes such as Sordariomycetes (1.3%), Agaricomycetes (0.6%), and Chytridiomycetes (0.5%) were also present consistently across all samples but in lower abundance. The greatest variation in abundance was found in Eurotiomycetes (2.1%–92.4%), Lecanoromycetes (0%–72.5%), and Leotiomycetes (0.16%–70.3%).

The composition of fungal communities showed limited correlations with chemical factors (Table S5, Supporting Information). The overall fungal community showed strong positive correlations with Fe and P (r = 0.80 and 0.66, respectively). For individual fungal classes, *Eurotiomycetes* showed significant correlation with Fe (r = 0.63) and Na (r = 0.72), and *Lecanoromycetes* showed significant correlation with Fe (r = 0.51) and Mg (r = 0.78). *Sordariomycetes* was positively correlated with Si (r = 0.51) and Ca (r = 0.53). Metastats analysis to assess the substrate mineralogy effects was not performed on fungal communities due to lack of replicates per site or rock type.

# Database searches to identify microbes closely related to the lithic microbes found in Svalbard

All sequences assigned to bacteria were clustered into 1835 OTUs with an average of 354 OTUs per sample. Across all six rock samples, we obtained 414 fungal OTUs, with a range of 41-111 OTUs per sample. We investigated whether bacterial and fungal OTUs found in this environment also occurred in other environments. DNA sequences of the top three most abundant OTUs per sample were BLAST-searched against the GenBank database (www.ncbi.nlm.nih.gov). Bacterial major OTUs obtained in this study were highly affiliated (97.0%-98.7% DNA sequence similarity) to strains that originated from soil or rocks with an Arctic or Antarctic origin (Table 1). The abundant bacterial members of sandstone and limestone were associated with mainly uncultured lineages of bacterial phyla such as Actinobacteria, Acidobacteria, Cyanobacteria and Proteobacteria. In volcanic rocks such as granite and basalt, the majority of bacteria were affiliated to Actinobacteria, previously found in Antarctic stone, rock and soil of Greenland, and deserts including Atacama and Norwegian cold desert. The abundant OTUs from Travertine were affiliated to Deinococcus-Thermus originated from the Antarctic Dry Valleys.

A similar trend was also found in fungi. Although fungal major OTUs showed relatively lower levels of DNA sequence

similarity (93.2%–98.8%) to the sequences of the closest relatives in the database, most affiliated sequences originated from soil, rock and lichen in the Arctic and Antarctica (Table 2). Ascomycota were the most abundant and most diverse members of the fungal communities in these study sites. They were related to many fungal sequences from cold environments. Among the dominant Ascomycota in sandstone and travertine, Ramalina terebrata (belonging to the class Lecanoromycetes) and Polycauliona verruculifera (belonging to the class Lecanoromycetes) have already been detected in Antarctica and Iceland. Verrucaria in limestone was observed in cold environments (i.e. Iceland and Norway). Regarding the OTUs belonging to Leotiomycetes, Tetracladium and Helotiales found in Troll springs were closely affiliated to those detected in the Antarctic and Alaska.

#### DISCUSSION

In this study, the rock-inhabiting microbial communities of five different types of Arctic rocks were analyzed using culture-independent molecular methods. Possible associations between lithic microbial community and rock geochemistry were assessed using the Mantel test. The results showed that the rocks in Svalbard were capable of harboring diverse microbial communities, which were influenced by geographical features and rock types.

There was a certain level of variation in community composition between samples within the same rock type, but the difference was not larger than that between different rock types. For example, in bacterial community composition of limestone, DS10 has a higher relative abundance of Proteobacteria and Acidobacteria than DS3, while Actinobacteria relatively low in DS10. In travertine, L10, L11 and L12 showed similar community composition, whereas in L15, the relative abundance of Cyanobacteria was remarkably low. These difference may be due to the difference of chemical composition of rock, and microenvironment in which they are located. Chemical composition was distinct different according to rock types, but there was also a slight difference among the same type of rock (see Table S4, Supporting Information). The chemical composition and physical structure of rock are generally determined by the geologic processes by which it formed, and the both of them can vary within same type of rocks (Deer, Howie and Zussman 1992). These chemical properties of rock can impact rock-inhabiting microbial community (Uroz et al. 2009). In case of travertine, L15 was sampled far away from the troll spring with water source, unlike other rocks sampled around the troll spring. This may have led to a relatively low abundance of Cyanobacteria, which is affected by moisture content (Starke et al. 2013). For these reasons, microbial community composition in the same type of rock can be slightly different. Nevertheless, the significant differences of microbial community composition according to rock types are still worth considering.

Five bacterial phyla, Actinobacteria, Proteobacteria, Chloroflexi, Bacteroidetes and Acidobacteria, were dominant in all rock samples, but their relative abundance differed considerably according to the rock type. These phyla were often observed in other extreme environments, and a wide range of relative abundance variations of certain phyla across different regions or rock types has also been reported (Friedmann 1980; Wong et al. 2010). In this study, Actinobacteria in rock samples had the largest variation of the relative abundance among bacterial phyla, ranging from 11.0% to 83.8%. The microbial composition analysis

			Sequences	Closest sequence from NGBI	Cimilarity		
Locality	Lithology	OTU <sup>a</sup>	(%)	DB/Accession number	(%) <sup>b</sup>	Taxonomy <sup>c</sup>	Isolation source
Smithelva	Sandstone	DS1_05	8.52	Uncultured Actinobacterium clone MVH85/EU931059	98.4	Nocardioidaceae	Antarctic Dry Valley, mineral soils
		DS1_11	6.38	Uncultured Acidobacteria bacterium clone S 4/FI895045	98.4	Blastocatella	Antarctic Dry Valley, mineral soils
		DS1_18	6.34	Uncultured bacterium clone	97.5	Sphingomonas	Antarctic Miers
Smithelva	Sandstone	DS2_04	6.92	Uncultured bacterium clone	97.5	Acetobacteraceae	Mont Blanc, snow
		DS2_05	4.76	Uncultured Actinobacterium clone	98.2	Nocardioidaceae	Antarctic King
		DS2_26	3.94	Acetobacteraceae bacterium strain	98.2	Acetobacteraceae	Antarctic, soil
Stupbekken	Limestone	DS3_19	5.87	Uncultured bacterium clone 1A-E9FH3/HQ197559	98.7	Loriellopsis	Antarctic Dry Valleys, quartz rocks
		DS3_15	3.37	Uncultured alpha proteobacterium clone C_9/FJ490246	97.5	Sphingomonas	Antarctic Dry Valleys, rock
		DS3_28	2.79	Uncultured bacterium clone RamatNadiv02a11/JF295332	97.7	Ilumatobacter	Negev desert, soil
Stupbekken	Limestone	DS10_03	21.88	Uncultured bacterium clone VS12–69/JX258090	97.7	Chroococcidiopsis	Arctic Svalbard Troll spring, rock
		DS10_04	13.99	Uncultured Acetobacteraceae bacterium clone B9_37/AM940870	97.0	Acetobacteraceae	Arctic Ny-Ålesund, glacier moraine
		DS10_15	6.71	Uncultured endolithic bacterium clone SM_12_BAC/AB473921	98.1	Sphingomonas	Switzerland, central Alps, white rock
Troll spring	Granite	L7_10	14.68	Uncultured Arthrobacter sp. clone CNY_0 1652 /JQ401533	97.9	Pseudarthrobacter	Dry desert, soil crust
		L7_44	3.57	Nocardioides sp. G03 45–8/KF974316	97.3	Nocardioides	Arctic Northeast Greenland, soil
		L7_13	2.08	Arthrobacter sp. BAR81/KP717965	97.5	Arthrobacter	Antarctic Dry Valley, rock
Halvdanpiggen	Basalt	L23_13	11.70	Arthrobacter sp. BAR76/KP756683	97.7	Arthrobacter	Antarctic Victoria Land, stone
		L23_17	10.51	Uncultured Nocardioidaceae bacterium clone5B_17/HE861127	97.7	Nocardioidaceae	Norway, cold desert, rhizosphere soil
		L23_07	7.03	Uncultured bacterium clone 4B_04H/JX098493	98.2	Frankiales	Atacama desert 6000 m, mineral soil
Troll spring	Travertine	L10_01	9.35	Uncultured bacterium clone VS12–57/JX258077	97.0	Chroococcidiopsis	Arctic Svalbard Troll spring, rock
		L10_16	6.48	Uncultured bacterium clone VS12–50/JX258070	97.5	Pseudanabaenaceae	Arctic Svalbard Troll spring, rock
		L10_02	5.53	Uncultured Deinococcus sp. clone S_6/FJ895047	97.4	Truepera	Antarctic Dry Valleys, soil
Troll spring	Travertine	L11_02	8.22	Uncultured Deinococcus sp. clone S_6/FJ895047	97.4	Truepera	Antarctic Dry Valleys, soil
		L11_06	8.12	Leptolyngbya sp. ANT.L52.1/AY493584	97.5	Leptolyngbya	Antarctic microbial mat
		L11_01	7.78	Uncultured bacterium clone VS12–73/JX258095	96.8	Chroococcidiopsis	Arctic Svalbard Troll spring, rock
Troll spring	Travertine	L12_02	9.55	Uncultured Deinococcus sp. clone S_6/FJ895047	97.4	Truepera	Antarctic Dry Valleys, soil
		L12_08	8.61	Uncultured Cyanobacterium clone1C_21/HE860791	97.9	Chroococcidiopsis	Norway, cold desert, rhizosphere soil
		L12_01	7.56	Uncultured bacterium clone VS12–57/JX258077	97.0	Chroococcidiopsis	Arctic Svalbard Troll Spring, rock
Troll spring	Travertine	L15_14	9.76	Uncultured bacterium clone LNH_9_9_11_Pumice.78534/KM120111	97.5	Brevundimonas	Argentina, Lake Naheul Huapi, rock
		L15_23	6.92	Sporosarcina sp. PE.8/GU047418	97.8	Sporosarcina	Arctic permafrost wetland acidic soil
		L15_09	2.91	Uncultured Sphingomonadaceae bacterium clone3A_12/HE860905	98.4	Sphingosinicella	Norway, cold desert, rhizosphere soil

Table 1. Phylogenetic affiliation and isolation source of dominant bacterial OTUs obtained from rock samples.

<sup>a</sup>Representative 16S rRNA gene sequences of the top three most abundant OTUs per sample were BLAST-searched against the GenBank database (www.ncbi.nlm.nih .gov).

<sup>b</sup>Sequence similarity to its closest relative in the GenBank database

<sup>c</sup>Taxa names are represented at the taxonomic level identifiable by EzTaxon-e database (Kim et al. 2012).

Table 2. Phylogenetic affiliation and isolation source of dominant fungal OTUs obtained from rock samples.

Locality	Lithology	OTU <sup>a</sup>	Sequences per sample (%)	Closest sequence from NCBI nucleotide DB/Accession number	Similarity (%) <sup>b</sup>	Isolation source
Smithelva	Sandstone	DS1_04	24.8	Lecanorales/AY300842	97.6	Sweden, lichen
		DS1_05	21.5	Acarosporaceae fungus/LN810842	98.8	Norway, lichen
		DS1_06	11.8	Teloschistaceae fungus/KT291561	97.3	California, semiaquatic rock
Smithelva	Sandstone	DS2_02	91.0	Uncultured fungus/KC965541	95.7	Canadian High Arctic, soil
		DS2_10	1.5	Uncultured fungus/KC965931	97.3	Canadian High Arctic, soil
		DS2_31	1.1	Uncultured fungus/KC966351	99.6	Canadian High Arctic, soil
Stupbekken	Limestone	DS3_01	84.1	Verrucaria sp./FJ664858	98.2	Iceland, rock
		DS3_08	5.5	Uncultured fungus/KC966034	97.3	Canadian High Arctic, soil
		DS3_12	2.6	Thamnogalla crombiei/KJ559557	93.2	Iceland
Stupbekken	Limestone	DS10_01	45.5	Verrucaria sp./KF297206	99.4	Canadian High Arctic, soil
		DS10_03	39.6	Uncultured fungus/KC965115	93.5	Alaska Howe Island, soil
		DS10_20	2.3	Trapeliaceae fungus/EF489925	95.7	Antarctic King George Island, lichen
Troll spring	Travertine	L7_14	38.6	Uncultured fungus/KC966090	99.1	Canadian High Arctic, soil
		L7_27	16.9	Uncultured fungus/KC965406	99.1	Canadian High Arctic, soil
		L7_42	4.8	Uncultured fungus/KC965963	97.9	Alaska Franklin Bluffs, soil
Troll spring	Travertine	L11_07	70.2	Fulgensia sp./AF279882	97.6	Romania, lichen
		L11_01	18.5	Uncultured fungus/KC966368	98.5	Canadian High Arctic, soil
		L11_10	1.7	Uncultured fungus/KF296796	98.8	Canadian High Arctic, soil

<sup>a</sup>Representative 28S rRNA gene sequences of the top three most abundant OTUs per sample were BLAST-searched against the GenBank database (www.ncbi.nlm.nih .gov).

<sup>b</sup>Sequence similarity to its closest relative in the GenBank database.

showed that basalt from Halvdanpiggen had the highest proportion of Actinobacteria and that they dominated the bacterial population. In previous studies, Mg was found to influence the distribution of Actinobacteria communities in cold habitats (Bajerski and Wagner 2013). Among the elements present in basalt, Mg was at a higher level than that observed for other rock types. Indeed, Spearman's rank correlation analysis revealed a significant correlation between actinobacterial relative abundance and Mg content. In addition, *Rubrobacteraceae* in Actinobacteria were the most abundant group observed in basalt sample. *Rubrobacteraceae* embrace the genus *Rubrobacter* with three species that tolerate extremely high levels of ionizing radiation: R. *xylanophilus*, R. *taiwanensis* and R. *radiotolerans* (Suzuki *et al.* 1988; Carreto *et al.* 1996; Chen *et al.* 2004). This indicates that the ability to resist radiation may be important in this location.

In contrast to Actinobacteria, Proteobacteria and Bacteroidetes showed the lowest abundance in the basalt from Halvdanpiggen, despite being consistent in other samples. Although not all members of the Proteobacteria and Bacteroidetes phyla are copiotrophs, they are known to be copiotrophs that are typically found in nutrient-rich environments and grow in conditions with high levels of organic substrates (Fierer et al. 2012). In a study of basaltic glass from Iceland, Cockell et al. (2009) found that community dominated by Proteobacteria and Bacteroidetes in contrast to our results. However, the basalts examined by them are relatively copiotrophic environment compared with other types of volcanic rocks. In another study conducted in Savanna including basaltic soils, RughÖft et al. (2016) observed low relative abundance of Proteobacteria in oligotrophic environment. They explained the results based on oligotrophy-copiotroph category, which predicts that fast-growing bacteria (copiotrophs) prefer nutrient rich environments, while slower growing oligotrophs would thrive in oligotrophic environments. Consistent with these studies, the lower relative abundance of Proteobacteria and Bacteroidetes in Halvdanpiggen basalt samples may be

ascribed to oligotrophic environment of high-altitude volcanic habitats formed in that area.

Cyanobacteria have frequently been observed as members of rock-inhabiting bacterial communities in various cold deserts of the Arctic (Ehrlich 1998; Omelon, Pollard and Ferris 2006b; Omelon, Pollard and Ferris 2007; Ziolkowski et al. 2013). Cyanobacteria have been reported in Arctic endolithic habitats with a nearby water source, and their abundance was influenced by moisture content (Starke et al. 2013). Consistent with these studies, our data showed that the rock samples at Troll spring were more abundant and diverse than those from other study sites lacking a water source. Furthermore, Deinococcus-Thermus was most abundant in travertine samples from Troll spring, and the majority of the Deinococcus-Thermus sequences belonged to the family Trueperaceae. Predominance of Trueperaceae-associated sequences in travertine samples was in agreement with previous study by Starke et al., that also showed the high abundance of Trueperaceae members in Troll Spring (Starke et al. 2013).

In terms of correlation between rock elements and rockinhabiting bacterial community, although the paucity of rock samples restricted our statistical power, we were still able to observe that certain bacterial groups had a substrate preference (see Table S6, Supporting Information). They preferred carbonate rocks, and this result showed differential occurrence of some bacterial genera depending on contrasted mineralogical substrates (carbonate rock and non-carbonate rock). For example, a few bacterial genera that were preferentially abundant in carbonate rock, such as Rhodococcus and Sphingomonas, have been considered to play a potential role in carbonate mineral formation (Stoner et al. 2005; Rusznyak et al. 2012). Rhodococcus served as nucleation site for Ca-carbonate precipitation (Rusznyak et al. 2012) and Sphingomonas mediated the hydrolysis of urea, which resulted in the formation of carbonate (Stoner et al. 2005). Although the formation of carbonate biominerals by

these microorganisms remained elusive, this differential occurrence of bacterial genera depending on the rock's chemistry suggested that rock-inhabiting microbes had specific association to specific minerals. Uroz et al. (2009) proposed that the hypothesis of a so-called 'mineralosphere'. The hypothesis is that the ability of microorganisms to preferentially use inorganic nutrients released by surrounding soil (or rock) affects microbial community structure in their habitat. Gleeson et al. (2006) also showed that the structure of bacterial communities inhabiting granite significantly differed by the type of mineral inclusions, suggesting that mineral chemistry may be one of the important factors determining bacterial community composition. In addition, Cockell et al. (2009) showed that both Bacteroidetes and Actinobacteria isolates can be cultured from basaltic glass using crushed basalt glass (and its indigenous carbon sources) as the growth substrate, showing that there exists a core population of these organisms within the rocks that represent the metabolically active components. Our results are consistent with these studies showing that rock-inhabiting microbial community may be influenced by rock elements. However, further research will be needed to evaluate the rock elements as substrate potentials of microbes from the lithic environments.

Rock-inhabiting fungi are known to be ubiquitous in hard surfaces in extreme climates (Gorbushina 2007; Gorbushina and Broughton 2009). They are well adapted to nutrient-poor and dry habitats where they are particularly successful colonizers because of their restricted competition with other microbes and their extremotolerance (Gorbushina 2007). Rock-inhabiting fungi have also been reported in both igneous and sedimentary rocks, including siliceous types (silica, silicates and aluminosilicates), sandstone, granite, limestone, marble and gypsum (Staley, Palmer and Adams 1982; Ehrlich 1998; Sterflinger 2000). In this study, five fungal classes, Dothideomycetes, Eurotiomycetes, Lecanoromycetes, Leotiomycetes and Sordariomycetes, were predominant across rock samples, with all classes belonging to Ascomycota. Goordial et al. (2016) found that Dothideomycetes (order Capnodiales and Pleosporales) and Eurotiomycetes (order Chaetothyriales) dominate the Dry Valley, Antarctica. Selbmann et al. (2013) also documented dominant fungal members on samples obtained from various locations in Northern and Southern Victoria Land, Antarctica. Recovered 78 fungal sequences were dominated by Dothideomycetes (Orders Capnodiales, Dothideales, Myriangiales and Botryosphaeriales) and Eurotiomycetes (Order Chaetothyriales). In the Arctic, Ziolkowski et al. (2013) found that recovered fungal sequences from the endolithic region were dominated by lichenized Ascomycota (35% endolithic Verrucariales). In the Atacama Desert, a total of 81 rock-associated fungal isolates were classified as 29 Ascomycota taxa by Gonçalves et al. (2016). These Ascomycota taxa consist of classes Dothideomycetes, Sordariomycetes, Eurotiomycetes and Leotiomycetes. Likewise, rockinhabiting fungal communities in some studies are dominated by various fungal members belonging to the Ascomycota, although the relative abundance in lower level taxa varied depending on the study sites. These similar pattern seems to be due to their ability to survive in extreme environments. Several studies have revealed that the ability of microfungi to grow in rocky substrates was a polyphyletic trait, based on the assessment of rock-inhabiting fungi in two different classes of Ascomycota, namely Dothideomycetes and Eurotiomycetes (Sterflinger, de Hoog and Haase 1999; Gueidan et al. 2009; Ruibal et al. 2009). These classes are known to be polyextremophilic and are found as parasymbionts (symbionts to lichen) in Antarctic lithic habitats (Selbmann et al. 2005). Eurotiomycetes and Dothideomycetes include the 'black yeast' fungi, which are melanized

and are known for their desiccation and UV resistance (Selbmann et al. 2005; Ruibal et al. 2009), and may play an important role in community protection from excessive UV radiation. In addition, Lecanoromycetes, Leotiomycetes and Sordariomycetes contain species known as producers of some of the most important fungal secondary metabolites (Lawrey 1986; Molnar and Farkas 2010). Secondary metabolites are considered to have important biological and ecological roles. Several secondary metabolites (including atranorin, calycin, pinastric acid, pulvinic acid, rhizocarpic acid, usnic acid and vulpinic acid) have strong UV absorption abilities and might function as filters for excessive UV-B irradiation (Lawrey 1986; Molnar and Farkas 2010). Thus, these characteristics of fungi could be a possible reason for the predominance of those fungal classes in this study sites. These rock-inhabiting fungi may also be associated with the weathering of rocks by enhancing mineral dissolution and diagenesis. The weathering activity of fungi may occur as a result of oxidative or reductive attack of reactive mineral constituents, e.g. Mn and Fe (Delatorre and Gomezalarcon 1994). Indeed, there was a significant correlation between the whole fungal community and Fe. Mineral-weathering ability of fungi can also appear with bacteria, such as lichen. Lichen directly or indirectly impact mineral weathering process via various activities such as mineral disaggregation, hydration, dissolution and secondary mineral formation (Banfield et al. 1999). Furthermore, certain bacteria can alone cause mineral weathering by interacting with various minerals (Uroz et al. 2009; Gadd 2010). These microorganisms related mineral weathering might provide various nutrient elements for life in lithic environments (Gadd 2010). Further study will be needed to combine functional and taxonomic investigations for microbial weathering in lithic environment.

The effects of geographic distance on the lithic community structure are confounding for both bacterial and fungal communities. Some samples with close proximity to one another shared more similar microbial lineages, while lithic communities collected from geographically distant locations also clustered together (see Fig. S2, Supporting Information). It is possible that the varying physicochemical and environmental factors between rock types will interact in a complex manner to more influence lithic microbial communities than geographical distance. For instance, microbial community influenced by their ability to use the nutrient elements released by surrounding habitats (Uroz et al. 2009). However, more extensive sampling at multiple spatial scales is necessary to assess the biogeographical pattern in lithic microbial communities.

Among the OTUs detected in this study, the top three dominant OTUs per rock sample were closely affiliated to microorganisms inhabiting the soil or rock from other extreme environments. Geographically, microbes closely related to those major bacterial OTUs have mainly originated from the terrestrial environment of the Arctic and Antarctica, and some are found in deserts (e.g. Atacama Desert in Chile and Negev Desert in Israel). In fungi, taxa closely related to those major fungal OTUs are frequently found in Canadian High Arctic and Northern Alaska, and some occurred in sub-Arctic regions and Antarctica (Table 2). When we compared a query sequence with NCBI database using Blast, one or more different sequences showed the same similarity to the query. For example, OTU DS10\_03, the most abundant OTU (21.88%) among all rock samples, showed the highest sequence similarity to only one other sequence. On the other hand, six different sequences showed the same similarity to OTU L10\_01, and one of them was selected and listed in Table 1. In the case of OTU L10\_01, the close sequences were

originated from Arctic Svalbard, Canadian High Arctic, Antarctic microbiological mat, and hyper-arid polar desert such as Antarctic Dry Valleys. The abundant bacterial OTU associated with rock samples from high Arctic polar desert in this study showed the significant sequence similarity to bacteria from cold and/or arid environments. In previous biogeographic and phylogenetic studies of rock-inhabiting microbes, similar microbial assemblages were found in rock environments, even if they were located in different continents (Büdel 1999; Fajardo-Cavazos and Nicholson 2006; Gueidan et al. 2008). An efficient mechanism of dispersal, most probably wind-mediated, may have led to colonization spanning different habitats (Gorbushina 2007; Gorbushina and Broughton 2009). Consistent with previous studies, this study showed that the abundant OTUs had the closest match to sequences previously reported from polar and arid environments, especially rock and soil. None of the detected OTUs were affiliated to microorganisms living in terrestrial deep subsurface environment which is one of the largest lithic ecosystems (Whitman, Coleman and Wiebe 1998; McMahon and Parnell 2014). It is not surprising because the unique geophysical and geochemical conditions of the terrestrial deep subsurface are different from those of terrestrial surface environments. For example, deep subsurface environments are commonly anoxic because these environments are isolated from the atmosphere (Lovley and Chapelle 1995). Also, with an increase in depth from surface, photosynthetically derived nutrients come to be limited (Pedersen 2000). These unique conditions lead to unique microorganisms in deep subsurface environments (Pedersen 2000; Miettinen et al. 2015; Rempfert et al. 2017). For these reasons, it is reasonable that OTUs detected in our study are not associated with microorganisms found in the terrestrial deep subsurface environments.

Taken together, the ability of rock-inhabiting microbes to survive in varied environments depends on key features such as oligotrophy, resistance to various stresses, and diversity of growth forms. These key features related to the phylogenetic commonality of rock-inhabiting microbial communities in various extreme habitats. For example, Pseudarthrobacter sulfonivorans Ar51 that matched most closely to the representative sequence of OTU10 in sample L7 (97.75% 16S rRNA sequence similarity) is a psychotrophic bacterum that can produce trehalose as the osmotic stress protector and aquaporin giving freezetolerance under rapid-freezing conditions (Zhang et al. 2016). Anabaena cylindrica, Phormidium persicinum, Scytonema hofmanni and Leptolyngbya nodulosa belonging to Cyanobacteria in the closest species list have the ability to secrete extracellular polymeric substances (EPS) (Li, Harding and Liu 2001; Pereira et al. 2009; Baldev et al. 2015). The synthesis of EPSs contributes to a structurally stable and hydrated microenvironment, as well as chemical/physical protection against biotic and abiotic stress factors in extreme environments. In addition, Sporosarcina globispora (97.42% sequence similarity with the representative sequence of OTU23 in sample L15), formerly known as Bacillus globisporus, is a spore-forming bacterum (Larkin and Stokes 1967). Considering the ability of life to survive in extreme environmental stresses, bacterial spores stand out as the epitome of longevity, tenacity and persistence.

In conclusion, this study examined the microbial composition and diversity of lithic communities inhabiting different rock types in Svalbard. Lithic communities in this region were dominated by stress-tolerant members of bacteria and fungi, and major microbial lineages were closely related to those frequently found in other cold and arid environments, supporting the hypothesis by Walker and Pace of a global meta-community uniquely adapted to the lithic habitat (Walker and Pace 2007a). The insights expand our knowledge of what communities dominate various rock types and what microorganisms are common in cold desert. Ongoing work examining site-specific factors such as substrate chemistry, microclimate, and host rock structure (e.g. grain size and translucence) will help clarify how the composition and diversity of lithic microbial assemblages have evolved in this high Arctic cold desert habitat.

## SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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