ENDOLITHIC MICROBIAL COMMUNITIES OF DIFFERENT TYPES OF ROCKS IN THE NORWEGIAN HIGH ARCTIC

Korea Polar Research Institute

Y. H. Choe, M. Kim, J. S. Woo, M. J. Lee, Y. K. Lee*
Korea Polar Research Institute
yhchoe@kopri.re.kr, yklee@kopri.re.kr*

Introduction

Extreme environmental conditions, such as Antarctica or the Arctic, are thought to test the limits of life. The extremely low temperatures, dry atmospheres, strong UV-radiation isolation, low nutrient availability or long periods without sunlight have not been obstacles in the adaptation and proliferation of several different types of microorganisms. Microorganisms living in these environments often seek protection from environmental stresses, with one sheltered habitat found within rocks. The microbial colonization in rock was influenced by the 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. In this study, we examined the bacterial and fungal endolithic community structure of different rock samples collected from four sites in Svalbard (Fig. 1). The main objectives of this work were (1) to investigate major members of bacteria and fungi colonizing the endolithic environment of Svalbard in the Arctic, and (2) to compare the endolithic microbes living other habitats.

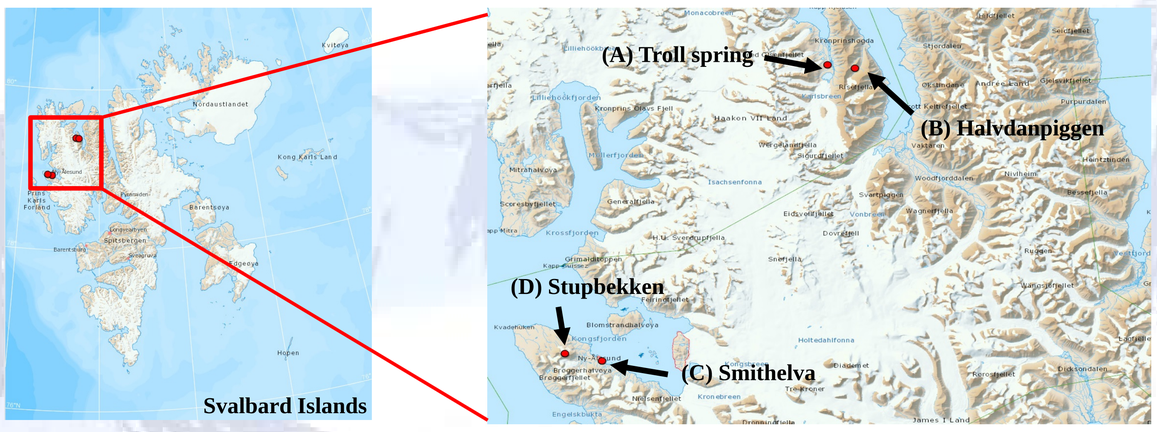


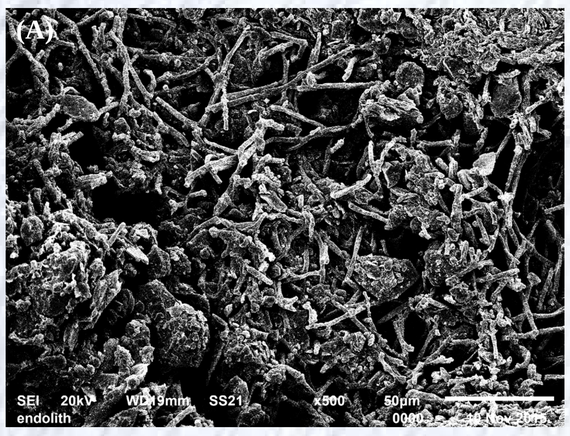
Figure 1. Landscape of sampling sites (A) Troll spring, (B) Halvdanpiggen, (C) Smithelva, (D) Stupbekken

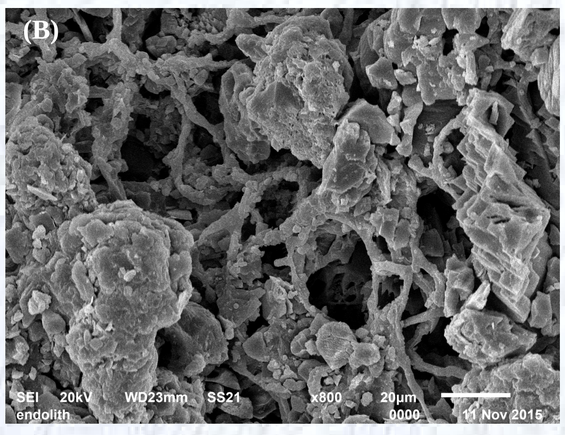
Materials and Methods

Rock samples were collected in August 2014 at four sites in Svalbard, Norway. Samples were collected with hardened steel chisels that were flame sterilized in the field with 100% ethanol. Rock samples at each site were collected into sterile, plastic tubes and bags, and transported to the laboratory in icebox keeping under ~4ºC. For scanning electron microscopy (SEM), rock pieces were sputter-coated with gold and examined with scanning electron microscope at 20 kV. Sequences generated from pyrosequencing of bacterial 16S rRNA gene amplicons were processed using the mothur pipeline. Statistical analyses were performed using the vegan R package.

Results

In field fracturing of rock samples revealed possibility of the presence of endolithic communities in several points. Scanning electron microscopy (SEM) analysis of the rock samples clearly revealed a diversity of microbiological structures, including both bacteria and fungi(Fig. 3). Hyphal structures were clearly visible on surfaces of all rock types, as well as microcolonial fungi (data not shown). The fungi are characterized by spindle-shaped swellings along the hyphae, and by spherical swellings inter-connected with the hyphae. Both rods and cocci were clearly visible in Fig. 3C and 3D. Figure 3C contained rod-shaped bacteria, approximately 1 µm in length, while Fig. 3D possibly contained rod-shaped bacteria and a clump of micro colonial fungi.





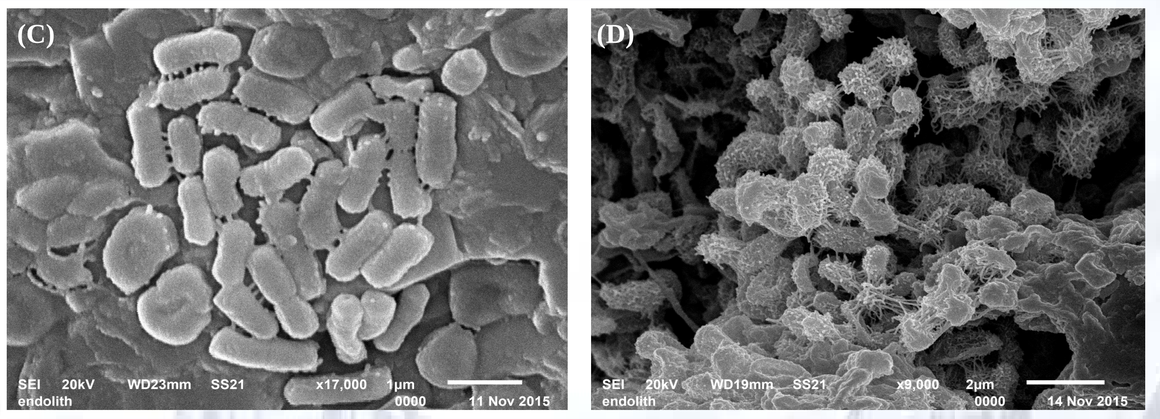


Figure 3. SEM micrographs of endolithic microorganisms. (A) and (B) images showing fungal hyphae on fractured rock. (C) and (D) SEM Images showing bacteria aggregates on fractured rock.

Both bacterial and fungal communities showed slight variation between different rock types. Bacterial communities were dominated by *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, *Bacteroidetes*, and *Acidobacteria*. Fungal communities consisted of *Eurotiomycetes*, *Lecanoromycetes*, *Dothideomycetes*, and *Leotiomycetes* (Fig. 4). Blast result revealed that dominant endolithic OTUs in this environment are highly affiliated with those found in soils or rocks of Arctic and Antarctica, suggesting 'niche conservatism' occurs at a global scale (Table 1).

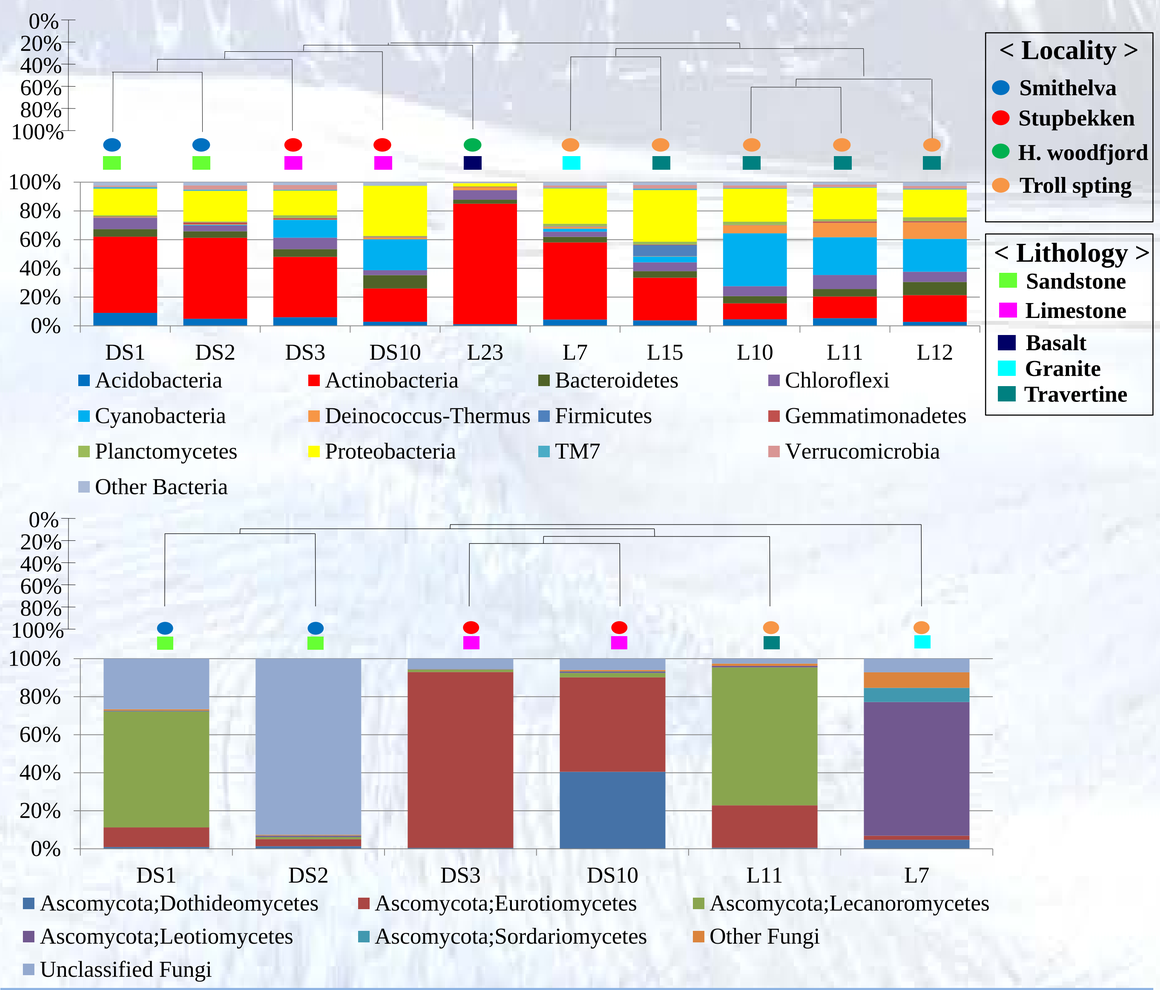


Figure 4. (A) Clustering pattern of rock samples based on bacterial phyla composition (B) Clustering pattern of rock samples based on Fungal classes composition

Bacterial OTU	The closest sequence from NCBI nucleotide database/Accession number	Percent Identity (%)	Isolation source
DS1_05	Uncultured *actinobacterium* / EU931059	98	Antarctic Dry Valley, mineral soils
DS2_04	Uncultured *Acetobacteraceae* bacterium / AM940870	97	Arctic Ny-Ålesund, glacier moraine
DS3_19	Uncultured bacterium / HQ197559	98	Antarctic Dry Valleys, quartz rocks
DS10_03	Uncultured bacterium / JX258090	98	Arctic thermal spring, endolith
L7_10	*Arthrobacter* / KP756677	98	Antarctic Victoria Land, stone
L10_01	Uncultured *cyanobacterium* / KM112123	97	Antarctic microbial mat
L11_02	Uncultured *deinococcus* / FJ895048	97	Antarctic Dry Valleys, soil
L12_02	Uncultured *deinococcus* / FJ895047	97	Antarctic Dry Valleys, soil
L15_14	Uncultured bacterium / KM120111	98	Argentina: Lake Naheul Huapi, rock (pumice)
L23_13	*Arthrobacter* / KP756683	98	Antarctic Victoria Land , stone
Fungal OTU	**The closest sequence from NCBI nucleotide database/Accession number**	**Percent Identity (%)**	**Isolation source**
DS1_04	*Ramalina terebrata* / EU161239	96	Antarctica, lichen
DS2_02	Uncultured fungus / KC965541	96	North American Arctic, soil
DS3_01	*Verrucaria* / FJ664858	98	Iceland, rock
DS10_01	*Verrucaria margacea* / KM243204	98	Norway, rock
L7_14	*Tetracladium* / AB752249	99	Antarctica
L11_07	*Polycauliona verruculifera* / JQ301564	95	Iceland

Table 1. Phylogenetic Affiliation of the Dominant OTUs Obtained from Pyrosequencing Analysis of Rock Samples of the Svalbard

Conclusions and Implications

Although harsh conditions of Arctic have been thought to limit microbial diversity and richness within endolithic communities, we present data of Arctic endolithic community that is diverse and abundant. Our results show that various rocks in Svalbard are capable of harboring diverse microbial communities, despite the inhospitable habitats. Our study also provide insight into geobiological processes that shape the biosphere and help us understand the cold and dry environments possibly elsewhere in the Solar System.

References

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