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# Microbial communities associated with Antarctic snow pack and their biogeochemical implications

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ABSTRACT

Snow ecosystems represent a large part of the Earth's biosphere and harbour diverse microbial communities. Despite our increased knowledge of snow microbial communities, the question remains as to their functional potential, particularly with respect to their role in adapting to and modifying the specific snow environment. In this work, we investigated the diversity and functional capabilities of microorganisms from 3 regions of East Antarctica, with respect to compounds present in snow and tested whether their functional signature reflected the snow environment. A diverse assemblage of bacteria (Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Deinococcus-Thermus, Planctomycetes, Verrucomicrobia), archaea (Euryarchaeota), and eukarya (Basidiomycota, Ascomycota, Cryptomycota and Rhizaria) were detected through culture-dependent and –independent methods. Although microbial communities observed in the three snow samples were distinctly different, all isolates tested produced one or more of the following enzymes: lipase, protease, amylase, β-galactosidase, cellulase, and/or lignin modifying enzyme. This indicates that the snow pack microbes have the capacity to degrade organic compounds found in Antarctic snow (proteins, lipids, carbohydrates, lignin), thus highlighting their potential to be involved in snow chemistry.



## 1. Introduction

Snow and ice cover roughly 11% of the Earth's surface area. Snow overlays majority of the Antarctic continent (Goodison et al., 1999) and exerts an important control on the climate (Knight, 1999). The snow pack is not only a critical component of the global climate system, but is now recognized as a biome that constitutes dynamic reservoirs of microorganisms, organic material, and nutrients (Anesio and Laybourn-Parry, 2012; Stibal et al., 2012). The Antarctic snow pack harbours diverse, active and viable microbial populations that represent almost all the major phylogenetic groups (Carpenter et al., 2000; Fujii et al., 2010; Lopatina et al., 2013; Michaud et al., 2014; Yan et al., 2012). Recently, molecular approaches have identified specific functional genes, such as genes involved in nitrogen cycling, sulfur metabolism, carbon and nutrient recycling in the microbial communities inhabiting snow and ice (Larose et al., 2013; Shtarkman et al., 2013; Simon et al., 2009). Resident microbial communities are now believed to be actively impacting the nutrient dynamics, albedo and hydrochemistry of snow ecosystems (Larose et al., 2013). Interest in the structure, dynamics and functioning of these microorganisms is increasing because of their possible role in regulating regional and global biogeochemical cycles. Microbial mediated processes have a significant effect on the composition and abundance of nutrients in supraglacial environments (Hodson et al., 2008). Recent studies on carbon fluxes have shown that the supraglacial microbial communities may be playing an important role in carbon cycling through the production and consumption of dissolved organic matter (DOM) (Anesio et al., 2009; Hodson et al., 2010; Skidmore et al., 2000; Yallop et al., 2012).

Glacier ecosystems accumulate organic carbon from diverse sources (Antony et al., 2014; Bhatia et al., 2010; Pautler et al., 2011; Stubbins et al., 2012), a substantial fraction of which is labile and bioavailable (Anesio et al., 2010; Foreman et al., 2007; Hood et al., 2009; Singer et al., 2012). Molecular fingerprints of DOM in Antarctic snow, specifically in the Princess Elizabeth Land region

*Abbreviations:* ITS, Internal transcribed spacer; PCR, Polymerase chain reaction; DOM, Dissolved organic matter.

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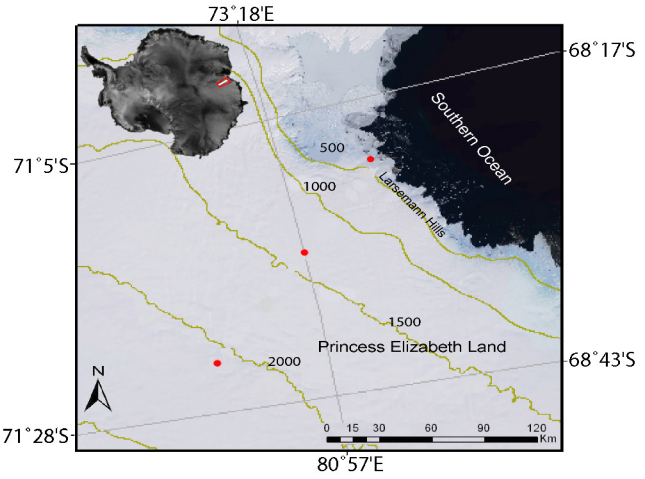


**Fig. 1.** Map showing snow sampling locations (red circles) along the Princess Elizabeth Land transect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of East Antarctica, indicate that the snow pack DOM is composed of material from *in situ* primary production as well as from deposition of organic material derived from the marine environment, secondary organic aerosols and long range atmospheric transport of terrestrial and anthropogenic organic matter (Antony et al., 2014). If DOM deposited on glacier surfaces serves as a source of nutrients for the supraglacial microbes, then the microbial community inhabiting the snow should be specially adapted to utilize these compounds. These organisms are therefore likely to possess the enzymatic machinery necessary to mineralize and utilize the limited resources available. But, very little information is available on the response of the microorganisms to the autochthonous as well as allochthonous carbon sources on the glacier surface and whether the supraglacial microbes have the enzymatic machinery to mineralize and transform this DOM. Therefore the aim of the present work was to explore the microbial diversity in the surface snow using culture-dependent and −independent methods and to investigate their functional capabilities by testing for their ability to produce enzymes specific to DOM compounds identified in the snow, and also to test the hypothesis that their functional signature reflects the snow environment.

## 2. Methodology

### 2.1. Surface snow sampling and processing

Microscopy based studies of snow from the Princess Elizabeth Land region of East Antarctica indicate that the surface snow in this region harbours abundant and diverse microbial communities (Antony et al., 2011). In order to better understand the microbial communities and their functional capacities, snow sampling was carried out at 3 locations along a transect from near the coast (coastal), 80 km (midland) and 180 km (inland) away from the coast (Fig. 1). Snow samples were collected aseptically into sterile WhirlPak bags. Before sampling, the surface snow at each site was thoroughly mixed using a sterile spatula in order to obtain a homogenously representative sample. In order to avoid contamination, the top few cm of the snow was removed using a sterile scoop before collecting the snow from beneath (about 20 cm depth). The ambient air temperature at these sites throughout the year ranges from −1 °C to −32 °C with a mean annual temperature of −16 °C (data available through the European Centre for Medium-Range Weather Forecasts). Table S1 presents data of chemical composition of snow at each sampling site.

### 2.2. Enrichment and isolation of native microorganisms

Snow was melted in sterile conditions at 4 °C. To check for bacterial contamination in the laboratory, a control consisting of sterile ultrapure water (Milli-Q water) was processed in a similar manner as that of the samples. To obtain a broad range of microbes, several media were used based on previous reports of successful recovery and isolation of stressed bacteria from polar ice samples (Amato et al., 2007; Christner et al., 2000; Reddy et al., 2000). Hundred microlitres of the snow melt-water was directly plated onto solid R2A media, Antarctic bacterial medium, dilute (1, 10 and 25% (w/v) of original strength media) tryptone soy agar, dilute nutrient agar and dilute Zobell marine medium. The plates were incubated at 4 °C and 20 °C. Un-inoculated controls were also maintained. In addition, enrichments were carried out by inoculating 1 ml aliquot of the melted snow sample, as well as the control into 9 ml of 1, 10 and 25% (w/v of original strength) media such as tryptone soy broth, nutrient broth, Zobell marine broth, Antarctic bacterial medium, R2A and R3A media. The tubes were incubated at 4 °C and 20 °C in the dark and examined daily. Blank media controls were prepared and incubated parallely with inoculated samples. On observation of growth, 0.1 ml was plated onto the corresponding solid medium for isolation of colonies. Un-inoculated controls were also plated on the respective solid medium. Morphologically distinct colonies were picked up and streaked onto the same medium to obtain single, pure colonies. The purity of the colonies was verified by gram staining before sub-culturing. Optimal temperature for growth was determined on dilute Luria Bertani broth (25% w/v of the original strength of 25 g $l^{-1}$) at temperatures ranging from 0 to 30 °C with an increment of 5 °C by measuring the optical density at 600 nm.

### 2.3. Genomic DNA extraction from isolates, PCR amplification and sequencing

Eighty two pure isolates (morphotypes) were selected based on differences in colony morphology, pigmentation, and media of isolation. Genomic DNA was extracted from each isolate using the UltraClean Microbial DNA isolation kit (MoBio). Bacterial 16S rRNA gene and the internal transcribed spacer (ITS) regions of fungal rDNA were amplified using the primers 27F/1492R (Lane, 1991) and ITS5/ITS4 (White et al., 1990), respectively. The 50 μl Polymerase chain reaction (PCR) reactions contained 25 μl 2X Taq PCR Master Mix (Promega), 15 pmol of each primer, and 2 μl of template DNA. After an initial denaturation at 95 °C for 2 min, 30 cycles of amplification consisting of: 95 °C for 30 s, 55 °C for 1 min, and 72 °C for 1.5 min; final extension at 72 °C for 10 min, were performed for primer pair 27F/1492R and 30 cycles of: 95 °C for 30 s, 50 °C for 1 min, and 72 °C for 1 min; final extension at 72 °C for 10 min, were performed for primer pair ITS5/ITS4. PCR products were evaluated by agarose gel (1%) electrophoresis, purified using the UltraClean PCR Clean-Up Kit (MoBio), and sequenced using an Avant 3100 gene analyser (Applied Biosystems). The 16S rRNA sequences obtained (~1 Kb and covering the V3–V5 region) and the ITS region of fungal 18S rRNA genes (~0.5 kb) were assembled and edited using DNA Baser Sequence Assembler v4 (Heracle Software, Germany), before sequence alignment. Taxonomic assignments were performed using EzTaxon-E (Kim et al., 2012). The threshold of ≥98.7% similarity was used for the operational definition of species (Stackebrandt and Ebers, 2006). For cases where an identity of <98.7% with a validated species was found, then these phylotypes were classified only to genus level, whereas those with <95% identity with the phylogenetically closest type strain were not assigned to a particular genus and were annotated at the family level (Sentausa and Fournier, 2013).