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Cyanobacteria inhabiting biological soil crusts of a polar desert: Sør Rondane Mountains, Antarctica

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ABSTRACT

Molecular and morphological methods were applied to study cyanobacterial community composition in biological soil crusts (BSCs) from four areas (two nunataks and two ridges) in the Sør Rondane Mountains, Antarctica. The sampling sites serve as control areas for open top chambers (OTCs) that were put in place in 2010 at the time of sample collection and will be compared with BSC samples taken from the OTCs in the future. Cyanobacterial cell biovolume was estimated using epifluorescence microscopy, which revealed the dominance of filamentous cyanobacteria in all studied sites except the Utsteinen ridge, where unicellular cyanobacteria were the most abundant. Cyanobacterial diversity was studied by a combination of molecular fingerprinting methods based on the 16S rRNA gene (denaturing gradient gel electrophoresis (DGGE) and 454 pyrosequencing) using cyanobacteria-specific primers. The number of DGGE sequences obtained per site was variable and, therefore, a high-throughput method was subsequently employed to improve the diversity coverage. Consistent with previous surveys in Antarctica, both methods showed that filamentous cyanobacteria, such as *Leptolyngbya* sp., *Phormidium* sp. and *Microcoleus* sp., were dominant in the studied sites.

In addition, the studied localities differed in substrate type, climatic conditions and soil parameters, which probably resulted in differences in cyanobacterial community composition. Furthermore, the BSC growing on gneiss pebbles had lower cyanobacterial abundances than BSCs associated with granitic substrates.

Introduction

Antarctica is the most isolated, coldest, windiest and driest continent on the planet [20]. Ice-free areas cover 0.34% of its total landmass [13], which includes nunataks, mountain peaks and other rock formations exposed above a glacier or ice sheet [33]. Biological soil crusts (BSCs), which consist of soil aggregates held together by communities of living organisms on the soil surface [4], are common in this type of environment, though the concept of BSC is not yet established in Antarctica, and most studies do not distinguish them from other types of soil communities [7]. The active growing season in Antarctica (when the soil surface temperature is above 0 °C and liquid water is available) lasts 25–75 days [10] resulting in limited diversity and abundance of photoautotrophic organisms, and low carbon and nitrogen concentrations in the Antarctic soil environment.

Cyanobacteria are usually the dominant components of the soil photoautotrophic community in Antarctic BSCs and they are the primary colonizers of poor Antarctic soils [6,16]. They are involved in important processes such as nitrogen fixation, moisture retention, soil stabilization and organic carbon accumulation [10]. Cyanobacteria are particularly well adapted to severe and variable conditions, having developed a wide range of strategies that allow them to minimize or avoid the harmful effects of harsh environments. Their ecological success can be partially attributed to the production of extracellular polymeric substances (EPS), which protect them from desiccation, and UV-screening pigments such as scytonemin [53].

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A recent review of Antarctic soil crusts [7] has provided an inventory of cyanobacteria identified to date based on morphological descriptions, with *Nostocales*, *Chroococcales* and *Oscillatoriales* shown to be present in all regions. In addition, microbial community composition in Antarctic soil crusts has been intensively studied in Victoria Land [1,18,28,31,57] and the Antarctic Peninsula [37,43,56,58]. Nevertheless, other Antarctic regions have received much less attention and knowledge of the cyanobacterial diversity of BSCs is still incomplete. Recently, a few studies have investigated the microbial diversity in different terrestrial habitats in the Sør Rondane Mountains (Dronning Maud Land, East Antarctica), a 250 km-long mountain range that rises up to more than 3000 m a.s.l. Fernández-Carazo et al. [17] and Obbels et al. [39] applied denaturing gradient gel electrophoresis (DGGE) to estimate the cyanobacterial diversity in soil crust and gravel samples from the Utsteinen ridge and nunatak, as well as a dry valley in the vicinity. In total, 18 operational taxonomic units (OTUs) were observed, of which 11 were present in several samples of the studied areas, which could be explained by easy dissemination within such a short distance. In addition, Obbels et al. [39] and Tytgat et al. [55], using high-throughput sequencing to study the bacterial diversity in various terrestrial habitats of the Sør Rondane Mountains, observed that cyanobacteria were abundant there. However, most analyses of the high-throughput data in these studies were carried out only at the phylum or order level.

Antarctic ecosystems are of particular interest due to the predicted effects of climate change, which are expected to cause changes in the soil microbiota [14]. In-field simulations of climate warming using open top chambers (OTCs) have been increasingly used to study the effects of climate change on the soil microbiome. These passive temperature-enhancing systems also modify several parameters, such as snow accumulation, moisture and wind [22]. Eight OTCs were installed in 2010 on flat platforms of the Utsteinen and Tanngarden ridges, and the Teltet and Pingvinane nunataks in the Sør Rondane Mountains. These geological formations are isolated and can be considered as oases surrounded by ice-covered terrain. In addition, visual inspections showed that there were more BSCs visible on granite substrates than on gneiss ones. Thus, the OTCs were placed on both types of substrates to understand better the role of the soil types. In the present study, a description of the soil parameters, diversity and abundance of BSC cyanobacteria is provided using morphological and molecular tools. A first DGGE survey resulted in unequal coverage and therefore additional high-throughput sequencing of the same segment of the 16S rRNA gene sequences (V3–V4) was performed to improve the molecular taxonomic data. It was hypothesized that the isolated locations and different environmental conditions, including substrate type, would result in different cyanobacterial community compositions. Furthermore, the samples were taken in the control areas of OTCs and, therefore, the present assessment constitutes baseline data for later comparisons with samples collected from inside the OTCs, which will provide insights on the effects of climate change on Antarctic soil cyanobacterial communities.

Materials and methods

Site description and sample collection

The studied sites were located in the western part of the Sør Rondane Mountains within a 30 km radius around the Belgian Princess Elisabeth Station (71°57′ S, 23°21′ E, 1372 m a.s.l.) (Fig. 1). The area has a continental climate with year-round subzero temperatures [41]. The Utsteinen ridge stretches from south to north for 700 m and is composed of massive coarse-grained granite. Samples were collected from the southern part of the ridge, approximately 300 m from the main building of the station (71°57′ S, 23°21′ E). Pingvinane is a range of six granitic nunataks located 20 km west from the station. Samples were taken on the west-southwest slope of the fourth nunatak from the northern end (72°00′ S, 22°59′ E), on a relatively flat slope composed of granite gravel. Tanngarden ridge is located 30 km west from the station. Soils and crusts formed on granite gravel were sampled in the wind scoop near the northeastern slope (72°01′ S, 22°56′ E). Teltet is a single nunatak located 8 km south-east from the station. Samples of gravel were collected on the flat surface on the northern ridge of the nunatak (71°59′ S, 23°29′ E) composed of gneiss pebbles, where a microbial mat instead of a developed BSC was observed. Pictures of the studied localities are shown in Supplementary Fig. S1.

In order to obtain microclimatic parameters, temperature and humidity sensors (Maxim i-Buttons®) were installed in each OTC and control site in 2010. Measurements were carried out in the period from 2010 to 2012 and the data from the sensors were recorded every 3 h. Replacement of the sensors was needed each year but could not be carried out when the OTCs were still under snow cover, which explains the absence of data during certain periods.

Soil crust samples were collected during the austral summer season in January 2010. For the measurements of soil chemistry and cyanobacterial cell biovolume, 15 representative subsamples, including bare soils, were collected from each site and mixed together in the field. For the molecular analyses, two to three soil crust samples were taken from each locality (11 samples in total) and they were analyzed separately. Soil crusts were placed into zip bags and shipped to the laboratory on dry ice. After sampling, the OTCs were installed at each site. Details regarding the implementation and monitoring of the OTCs will be published elsewhere (Namsaraev et al., in preparation).

Characterization of the soil crusts and soil nutrients

Soil crust coverage was estimated using digital photographs of the sampling sites within a 0.5 m × 0.5 m area. Chlorophyll *a* in the collected soil crusts was determined according to Namsaraev [36].

Analyses of soil physicochemical parameters were performed according to the methodology described in Czech and European Union standards (ISO 10390, ISO 10523, ČSN EN 27888, ISO 11465, ČSN EN ISO 11732, ČSN EN ISO 13395 and ČSN EN ISO 15681-1). In brief, water content was estimated after drying soils at 105 °C for 6 h. Soil pH was measured in 1 M KCl. Conductivity was evaluated in demineralized water. Macroelements (Ca, Mg, K, Na) were analyzed using a ContrAA® atomic absorption spectrometer (Analytik Jena, Jena, Germany). N–NH_4 and N–NO_3 concentrations were measured using a QuikChem® 8500FIA automated ion analyzer (Lachat Instruments, Loveland, USA). Phosphorus was detected as P–PO_4 using ascorbic acid–molybdate and a SHIMADZU UV-1650PC spectrophotometer. The percentage of total organic carbon (TOC) was determined by wet oxidation with acidified dichromate.

Cyanobacterial cell biovolume

For the estimation of cyanobacterial cell biovolume, light and epifluorescence microscopy was employed according to Kaštovská et al. [25]. A 1 g soil sample was diluted in 4 mL distilled water and the solution was transferred to a microscope slide, covered by a coverslip and observed by epifluorescence microscopy with 40× magnification (Olympus BX 51, Japan). A filter cube with green excitation at 510–550 nm (emission 590+ nm) was used to estimate cell abundance. Cyanobacteria were discriminated into three groups according to their cell morphology: unicellular, filamentous and heterocystous. Basic geometric equations for cylinders with

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