Microbial communities in different Antarctic mineral deposits characterised by denaturing gradient gel electrophoresis (DGGE)

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Summary A culture-independent method was used to assess the bacterial diversity in different mineral deposits of Livingston Island, Antarctic. One transect and four separate profiles were investigated. Total carbon and nitrogen were extremely low (< 0.23 %), whereas the water content ranged from 1.4% up to 35% with variations within single profiles. In two profiles permafrost was present in the deepest part (from 20 and 35 cm, respectively) of the sediments. DNA was recovered directly from mineral deposits and used as template for the amplification of bacterial 16S rRNA gene fragments. The mixture of 16S rRNA gene fragments was separated via denaturing gradient gel electrophoresis (DGGE). The DNA fingerprints showed a high number of bands that decrease with increasing depth, except for two single profiles, where no change within the profile could be observed.

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Introduction

Livingston Island, located at the tip of the Antarctic Peninsula (Figure 1), is characterised by an oceanic polar climate with temperatures above 0°C for 4 months per year and a mean annual precipitation between 400 and 500 mm (Vieira and Ramos, 2003, Serrano and López-Martínez, 2003). Under these conditions a soil formation can be observed (Blume et al., 1997) and lichens, mosses and some higher plants (*Deschampsia antarctica*, *Colobanthus quitensis*) are able to grow in this environment. Although the climate conditions are rough total microbial cell counts showed high numbers of microorganisms in the range of 10^7 and 10^9 cells g^{-1} dry weight (Bölter et al., 2002) in antarctic terrestrial habitats.

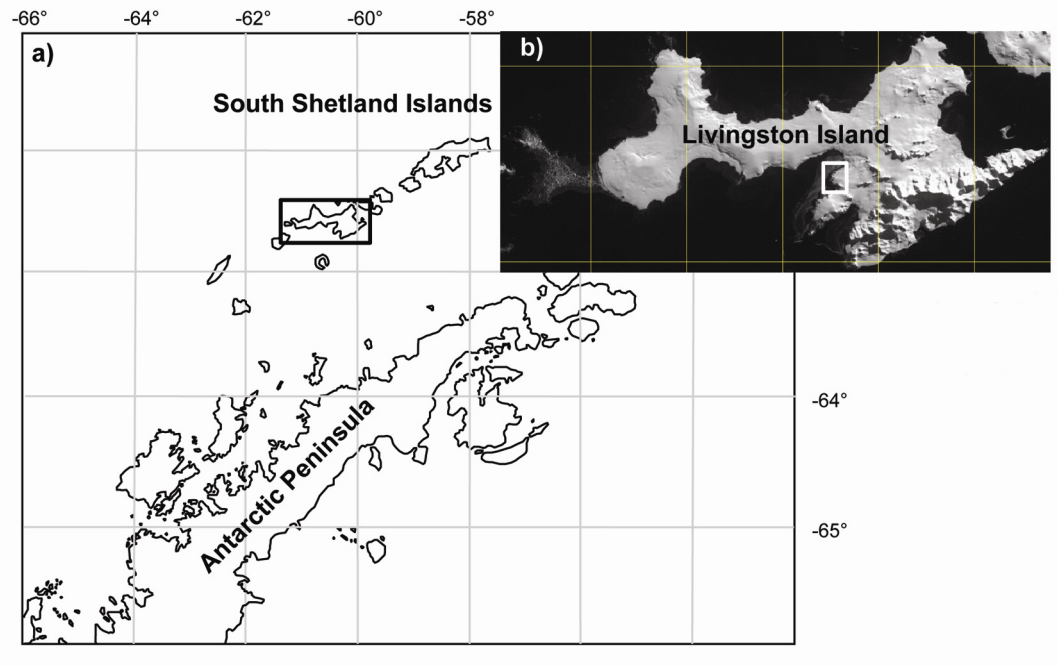

Figure 1. Maps of the investigation area: (a) South Shetland Islands north of the Antarctic Peninsula and (b) the study site on Livingston Island (62°38`S/60°21`W) (graphic by G. Schwamborn)

During the last few years the investigation of the composition of microbial communities in Antarctic habitats has been intensified (e.g. Bowman et al., 2000, Purdy et al., 2003, Aislabie et al., 2006, Gentile et al., 2006, Adams et al., 2006, Smith et al., 2006). Since cultivation-independent methods have become an important tool to investigate

environmental microbes, it is possible to analyze complex microbial communities in the face of diversity, abundance, ecology and their reaction on climate change.

In 2005 an expedition was carried out on Livingstone Island using the Bulgarian station as base for the investigations on microbial communities in extreme Antarctic habitats (Wagner et al., 2007). Typical sites for sampling were selected, using substrate characteristics, exposition, micro-climate and degree of site development as criteria, and described and sampled for further geochemical and microbiological investigations. The samples were transported in frozen condition to Germany.

Here, we investigated the bacterial community structure of different soil and sediments habitats located on Livingston Island by polymerase chain reaction (PCR) using a specific primer set followed by denaturing gradient gel electrophoresis (DGGE) to get a first insight in the diversity of bacteria existing under these conditions. Samples were taken throughout different soil profiles and mineral deposits from various depths to investigate the microbial community composition and their diversification within the different habitats.

Preliminary results

Soil description and grain size analysis

One transect and four separate profiles were sampled within walking distance of the Bulgarian station *St. Kliment Ohridski* (62°38`S/60°21`W) on Livingston Island. Two soil profiles were characterised by permafrost. The frozen ground leads to the formation of small puddles after rainfall. The investigated mineral soils showed all gravely sand texture, except the uppermost layer of the profile SP-A, which was silt dominated (Table 1). Moisture content of the soils ranged from 1.4% up to 35% and was partly very variable within the different profiles. The values of total carbon and nitrogen were extremely low with < 0.10 to 0.23% and < 0.10%, respectively.

Table 1 Geochemical and geophysical parameters of the investigated mineral deposits

Site location	Depth (cm)	Moisture (%)	Sand* (%)	Silt* (%)	Clay* (%)	Total C (%)	Total N (%)
T 1-2	0-5	11.7	62.7	17.5	19.9	0.11	< 0.10
	5-12	12.4	64.4	23.5	12.1	< 0.10	< 0.10
	12-24	8.2	70.4	16.0	13.5	< 0.10	< 0.10
T 1-3	0-9	7.9	72.3	21.6	6.1	0.13	< 0.10
	9-16	14.6	51.6	35.9	12.5	0.11	< 0.10
	16-23	7.4	83.1	12.3	4.6	< 0.10	< 0.10
	23-30	17.7	51.1	35.1	13.8	< 0.10	< 0.10
T 1-5	0-10	13.7	85.2	14.0	0.8	< 0.10	< 0.10
	10-20	17.7	87.9	10.2	2.0	< 0.10	< 0.10
	20-30	17.9	83.2	15.2	1.6	< 0.10	< 0.10
	30-40	22.4	79.5	20.1	0.4	< 0.10	< 0.10
	40-50	7.9	54.6	45.3	0.1	0.23	< 0.10
SP-A	0-5	28.0	38.3	60.6	1.1	< 0.10	< 0.10
	5-10	21.0	87.2	9.1	3.7	0.13	< 0.10
	10-15	13.7	81.0	14.5	4.5	< 0.10	< 0.10
	15-20	14.9	83.0	13.4	3.5	< 0.10	< 0.10
	20-25	17.6	90.5	7.7	1.7	< 0.10	< 0.10
SP-B	0-5	23.3	94.5	5.1	0.5	< 0.10	< 0.10
	5-10	35.0	92.2	6.5	1.3	< 0.10	< 0.10
	10-15	5.3	83.7	9.5	6.8	0.11	< 0.10
	15-20	5.6	82.9	13.1	4.0	0.10	< 0.10
	25-30	1.4	84.2	12.2	3.6	< 0.10	< 0.10
	30-35	4.8	50.9	37.0	12.1	< 0.10	< 0.10
SP-C	0-10	12.6	85.7	10.0	4.3	< 0.10	< 0.10
	10-20	6.8	52.6	32.3	15.1	0.12	< 0.10
	20-30	32.9	92.4	5.3	2.3	< 0.10	< 0.10
SP-D	0-5	15.4	92.7	6.7	0.6	< 0.10	< 0.10
	5-10	13.8	95.2	3.6	1.1	< 0.10	< 0.10
	10-15	13.2	94.5	4.3	1.2	< 0.10	< 0.10
	15-20	13.3	95.5	3.7	0.9	< 0.10	< 0.10

* part of the grain size fraction < 2mm

Denaturing gradient gel electrophoresis (DGGE)

DGGE patterns from amplification of DNA (Figure 2) showed differences in the vertical profiles and between the different sites. In the profiles of the transect the number of DNA bands decreased with depth. That could be also observed in the profiles SP-C and SP-D, whereas the number of DNA bands in the soil profiles SP-A and SP-B were more or less constant over the whole depth. The presence or absence of certain DNA bands within the profiles indicates a narrow spatial distribution of microorganisms.

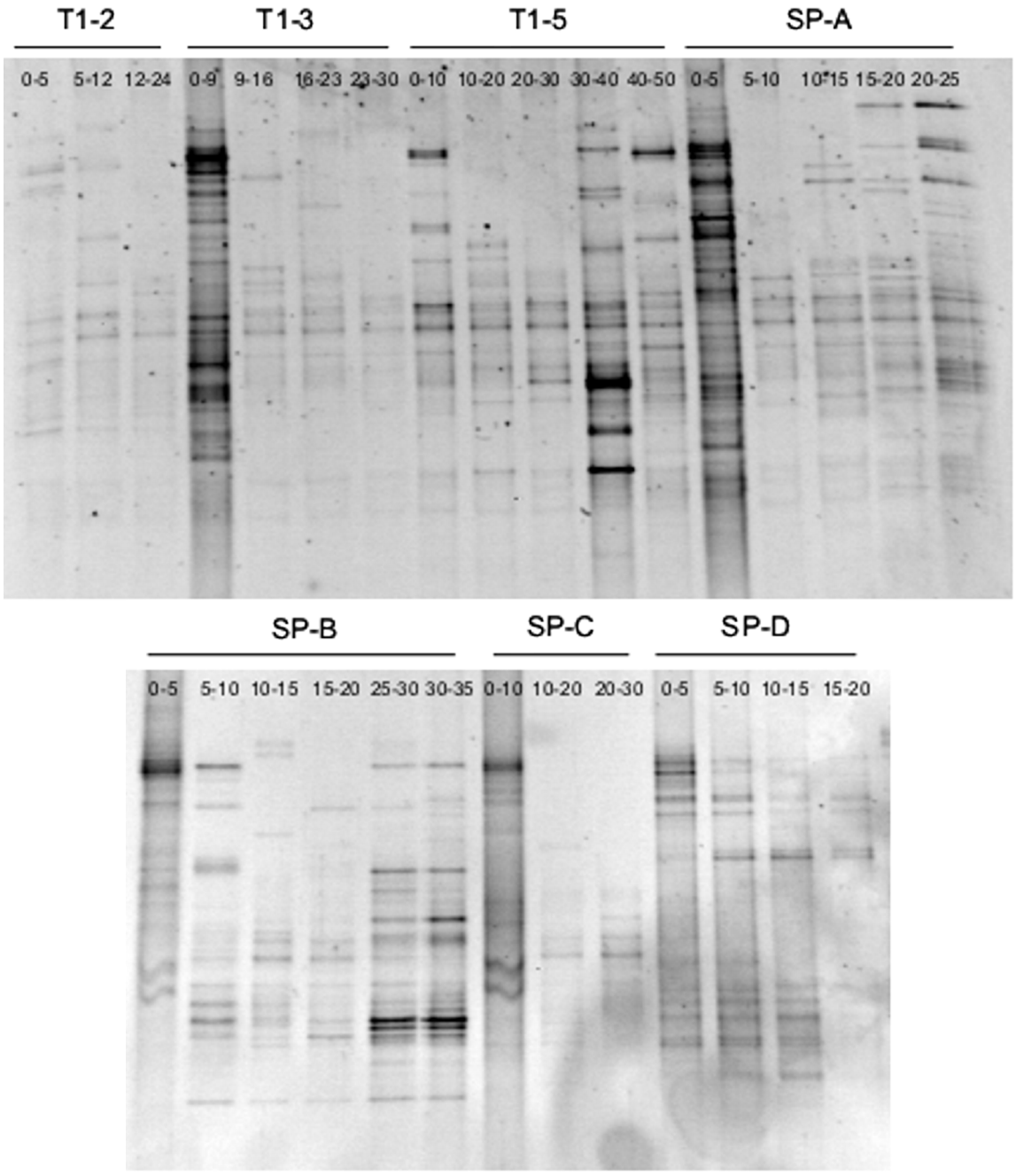

Figure 2. DGGE profiles of 16S rRNA gene amplicons from different mineral soil profiles from Livingston Island. T1-2, T1-3 and T1-5 represent three selected profiles of the investigated transect, whereas SP-A, SP-B, SP-C and SP-D stand for a single profile. Numbers in the DGGE picture indicate the sample depth.

Discussion

Although there was no or just low DNA recovery from the investigated soil and sediment samples a PCR product could be generated (data not shown) and the DGGE picture shows a high diversity in most of the samples. This was

surprising when we had a look on the nutrition status of these mineral sediments (Table 1). The main influence on heterotrophic microbial growth and activity in low-nutrient habitats, like in this study, is probably the availability of organic compounds, mainly C and N (Table 1, Barrett et al., 2006). Water is obviously not a limiting factor for bacteria in the investigated sediments from Livingstone Island due to relative high precipitation and temperatures above 0°C during the summer months. In contrast, cold dry mineral desert soils, which are more influenced by a continental climate, were not only restricted in carbon and nitrogen contents, but also in the availability of water (Aislabie et al., 2006, Smith et al, 2006).

Nevertheless, a wide diversity of bacterial members can be found in this hostile environment as could be seen on the DGGE pictures. It is conceivable that the ways of C and N cycling in cold antarctic habitats are very short and no accumulation of organic matter is possible when there is no large input of organic material and nutrients like in bird or seal colonies. In cold habitats without any organic nutrient input photosynthetic Cyanobacteria or chemolithotrophic microorganisms can be the only source of organic carbon. In arctic and alpine environments photosynthetic Cyanobacteria are related to a significant part of the bacterial community (Kastovská et al., 2005; Nemergut et al.,2005), whereas from sites along the Antarctic Peninsula the group of the Cyanobacteria make up only a minority of these bacterial communities (Yergeau et al., 2007). An advantage for microbial cells would be also the existence and expression of high-affinity nutrient uptake systems to have the possibility for growth under substrate-limited conditions (Vincent, 2000). Sequencing of DNA bands and phylogenetic analyses are in progress.

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