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Short Communication
T-RFLP Fingerprinting Analysis of Bacterial Communities in Debris Cones, Northern Victoria Land, Antarctica

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ABSTRACT

The debris cones known as Amorphous Glacier and Boulder Clay are located in an ice-free region in Northern Victoria Land, Antarctica, and differ in their isotopic composition, mechanisms of ice distribution, geological formation and age. However, to date it is not known if bacterial community profiles within ice and permafrost can be established for these environments, and then whether glaciological differences between the two areas would be reflected in the bacterial community composition. In order to gather first evidence for the bacterial communities in these glacial zones, we carried out terminal-restriction fragment length polymorphism (T-RFLP) analysis on the 16S rRNA gene using a universal bacterial amplification protocol on two permafrost cores. The DNA yields from ice-core samples ranged from 0.29 ng μL^{-1} in Amorphous Glacier to 88 ng μL^{-1} in Boulder Clay. Bray-Curtis cluster analysis suggested Boulder Clay bacterial profiles were similar to each other, but cluster separately from the Amorphous Glacier bacterial profile. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS: T-RFLP; fingerprinting; bacteria; debris cone; ice; permafrost; Antarctica

INTRODUCTION

Amorphous Glacier and Boulder Clay are two small ice-free areas located near Mario Zucchelli Station (Northern Victoria Land, Antarctica) and characterised by debris cones. Although in close proximity, Amorphous Glacier is above the Pleistocene grounding line and Holocene in age, whereas Boulder Clay is below the grounding line, with sediments likely of a glacio-marine origin and dated to the Late Pleistocene (Orombelli *et al.*, 1991).

Until a few decades ago, glacial formations and permafrost areas on the Antarctic continent have been seen as abiotic systems. However, new data are emerging that indicate microorganisms live within geological features, such as bacteria found in ice cores from Lake Vostok, Mizuho Base in the Enderby Land Mountains, and the Yamato Mountains in Dronning Maud (Christner *et al.*, 2001; Segawa *et al.*, 2010). These studies identified various genotypes within phyla Proteobacteria, Firmicutes, Cytophaga-Flavobacteria-Bacteroidetes, Actinobacteria and Deinococcus groups, which are known to contain bacterial taxa tolerant to cold and desiccation stresses (Christner *et al.*, 2000; Segawa *et al.*, 2010). Similarly, diverse bacterial compositions have been described from recent and ancient permafrost (Rivkina *et al.*, 2004, and references within). Knowledge of microbial life existing in ice does not only improve our understanding of the taxonomic diversity, richness and biogeography of cold-adapted microorganisms, but also assists in evaluating the metabolic requirements for survival and proliferation of life in the cryosphere, and in defining the actual limits of life.

Amorphous Glacier and Boulder Clay have been studied for their isotopic composition, mechanisms of ice distribution and geological formation (Guglielmin *et al.*, 1997; Gragnani *et al.*, 1998; French and Guglielmin, 1999; Guglielmin and

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French, 2004). However, it is unknown whether microorganisms occur in these sites. We therefore proceeded to test if bacterial DNA could be obtained from ice and permafrost cores of the Amorphous Glacier and Boulder Clay areas, and whether bacterial community profiles differ between these two distinct sites by way of terminal-restriction fragment length polymorphism (T-RFLP) analysis.

MATERIAL AND METHODS

Study Sites

The study area is located in Northern Victoria Land, Antarctica, close to Mario Zucchelli Station (74°41′36.96″S, 164°6′42.12″E). Samples were obtained from the debris cone of Amorphous Glacier (74°41′25″S, 164°00′E) and a frost-heaved mound 50 km away in Boulder Clay (74°44′45″S, 164°01′17″E). Amorphous Glacier is located west of Mario Zucchelli Station between 250 and 290 m above sea level. The summit of the cone is partially collapsed and its debris cover consists of 70–80 per cent light-grey granitic gravel. Ice within it represents congelation ice derived from groundwaters formed under different thermodynamic conditions (Guglielmin *et al*., 2002).

The Boulder Clay site is at 205 m above sea level and located south of Mario Zucchelli Station in an ice-free area (Guglielmin and French, 2004). The mean annual air temperature is −13.8°C, and the mean annual ground temperature is −16.1°C at the surface (2 cm depth) and −16.5°C at the permafrost table (30 cm), while in the deepest monitored layer (3.6 m, within the ice), the mean annual temperature is −17°C (Guglielmin and Cannone, 2012).

In the Boulder Clay area, an ablation till of late-glacial age overlies a body of buried glacier ice (Guglielmin *et al*., 1997; Gragnani *et al*., 1998; Guglielmin and French, 2004), and surface features include perennially ice-covered ponds with icing blisters and frost mounds, frost fissures, polygons and debris islands (French and Guglielmin, 2000). The age of the frost mound is younger than 1020 ± 70 ^{14}C yr BP, while the till that generally covers the surface of the Boulder Clay area is of Late Pleistocene age and attributed to the Ross Sea I glaciations (Orombelli *et al*., 1991). The analysed frost mound formed during the late Holocene, in the middle of a perennially ice-covered lake, which is located on the sublimation till, overlying the buried Pleistocene relict glacier ice (Guglielmin *et al*., 2009).

Ice Core Collection and Sample Preparation

Two ice cores were obtained during the austral summer in 1996 (Guglielmin *et al*., 2002). A 237-cm long ice core was extracted from the debris cone of Amorphous Glacier (AM). The Boulder Clay (BC) core was 375 cm long and sampled from a shallow perennially-frozen pond through the underlying sediment into the moraine-covered glacial ice. Both cores contained several distinct layers (Table 1). Amorphous Glacier was previously characterised chemically and isotopically (Guglielmin *et al*., 2002).

Samples for DNA extraction were aseptically cut from the ice cores at −40°C and stored on dry ice in a −40°C room. Internal parts of the cores were cut by an electric saw (repeatedly washed with ethanol) and stored in sterile Falcon tubes after the surface was washed with 70 per cent ethanol. BC samples contained a mixture of ice, stones and shells due its glacio-marine origin. These samples were crushed with an ethanol-washed hammer. Two duplicates from each sample were taken and stored in sterile Falcon tubes for further amplification and T-RFLP analysis.

DNA Extraction and Polymerase Chain Reaction (PCR)

Samples were thawed overnight at 4°C and always kept in the dark. AM samples were then filtered through a sterile 0.22 μm membrane (Millipore). The flow-through was collected in sterile Falcon tubes, lyophilised and resuspended in 1 mL sterile buffer. Filters were washed with 1 mL TNE buffer to recover bacterial cells. DNA was extracted from the filter and flow-through fractions using a protocol as previously described (Burns *et al*., 2004) with a modified incubation step with proteinase K (10 mg ml^{-1}) and SDS (10%) for 1 h, and finally resuspended in 50 μL sterile water. BC samples (400 μL) were added to 500 μL TNE buffer DNA extracted as described above and resuspended in 50 μL sterile Milli-Q water. All BC and AM samples were resuspended in a final volume of 50 μL sterile Milli-Q water. The presence of bacterial DNA, as well as the quality (presence of PCR inhibitors) of extracted DNA, was tested by universal bacterial 16S rRNA gene PCR using unlabelled 27F and 1494R primers.

To amplify the 16S rRNA gene fragment for T-RFLP analysis, PCR was performed with a labelled universal forward primer 27F 6-carboxyfluorescein (6-FAM) 5′ AGAGTTTGATCCTGGCTCAG) and a universal reverse primer 1494R (5′ TACGGCTACCTTGTTACGAC) in a 50 μL reaction (1X reaction buffer, 0.2 mM of each dNTP, 0.25 mM $MgCl_2$, 0.2 μM primers, 0.8 U *Taq* polymerase). After an initial denaturing step at 92°C for 2 min, 30 cycles of amplification followed (92°C for 20 s 50°C for 30 s, 72°C for 1 min), concluding with an extension step at 72°C for 7 min. Positive and negative controls were included in all PCR reactions. The DNA concentration was measured using a NanoDrop ND-1000 Spectrophotometer.

T-RFLP and Community Profiling

Approximately 150 ng of each FAM-labelled PCR product was digested with 6 U of the restriction endonuclease *Msp*I or 3 U *ScrF*I (New England Biolabs). Digestions were carried out in a total volume of 10 μL overnight at 37°C, following the manufacturer's instructions, and four replicates were analysed for each sample. The size of each terminal-restriction fragment (T-RF) was determined according to the GeneScan™ 1200 LIZ® size standard on an ABI 3730 Capillary Sequencer (Applied Biosystems Inc.) with an error of ± 0.5 base pairs (bp), and also analysed using Peak Scanner™ Software Version 1.0 (Applied Biosystems Inc).

Table 1 Amorphous Glacier and Boulder Clay samples: Ice-core section characteristics, DNA yields and 16S rRNA gene amplification results, and layer description.

Sample	Ice-core section (cm depth)	El. Cond. (μS cm^{-1} 20°C)	pH (20°C)	Cl^- (μeq L^{-1})	SO_4^{-2} (μeq L^{-1})	DNA (ng μL^{-1})	Amplification 16S rRNA gene	Layer description (Guglielmin *et al.*, 2002)
Amorphous Glacier								
AM-18	0–22	124	6.48	983.14	125.26	0.29	-	Active layer composed of loose sandy gravel with fine material increasing with depth
AM-3	75–79	19.5	5.73	155.45	10.77	2.44	+	Massive ice with high bubble density, elongated and big crystals; maximum chemicals concentration peaked in sinusoidal cycles every 60 cm in depth
AM-21	265–272	59.95	6.71	347.4	7.62	2.47	-	Massive ice with an intermediate bubble density, less elongated and smaller crystals; sinusoidal chemicals cycles were not present
Boulder Clay								
BC-1	0–15	n.d.	n.d.	n.d.	n.d.	88.02	+	Dynamic active layer in a small debris cone (0–30 cm depth changes)
BC-T	325–330	n.d.	n.d.	n.d.	n.d.	3.48	+	Massive ice and brine pockets within the ice (from a frost mound in a perennially frozen pond)
BC-B	370–375	n.d.	n.d.	n.d.	n.d.	9.29	+	Massive ice and brine pockets within the ice (from a frost mound in a perennially frozen pond)

- = No amplification; + = successful amplification; n.d. = no data. El. Cond. = Electrical Conductivity.

T-RFs were visualised as peaks in GeneScan™, which are characterised by width (bp) and height (arbitrary fluorescent units representing the relative abundance of a specific T-RF in a processed sample). Little background noise was present in the electropherograms. T-RFs more than 35 fluorescent units in intensity and present in at least two replicates were selected for further analysis (Liesack and Dunfield, 2004). For comparative analysis, T-RFs within an electropherogram were normalised to the total height of that sample (Dunbar *et al.*, 2000) and T-RFs with a relative height of less than 1 per cent of the total height were excluded from further analysis. T-RFs with peak heights determined to be off-scale by GeneScan™ were also excluded from further analysis, unless present in other replicates at lower heights, in which case these T-RFs were adjusted to the lower height value (Dunbar *et al.*, 2001). Bray-Curtis analysis was performed on presence-absence data using PAleontological STatistics program (PAST) (Hammer *et al.*, 2001). The Venn diagram was calculated with the online program Venny (Oliveros, 2007).

RESULTS AND DISCUSSION

Molecular fingerprinting analysis based on the bacterial 16S rRNA gene allows us to determine the presence of bacteria in environmental samples and their community profiles (Marsh *et al.*, 2000). We obtained DNA with concentrations ranging from 0.29 to 88.02 ng μL^{-1}, with the highest concentration from sample BC-1 (Table 1). DNA yields and bacterial cell counts would, however, be required to determine if the different DNA concentrations are due to changes in the distribution of bacteria in the ice cores. The partial 16S rRNA gene was successfully amplified from the samples BC-1, BC-T, BC-B and AM-3. Reasons for the failure of any amplification from the samples AM-18 and AM-21 could be due to a combination of low DNA concentration and degraded DNA (Rivkina *et al.*, 2004), as we did not detect PCR inhibition in the extracted nucleic acids.

T-RFLP analysis identified 18 T-RFs from *Msp*I and *ScrF*I digestions. Four T-RFs were found in all sites. There were 11 unique T-RFs and the majority were detected in the AM sample (Figure 1A). The BC-B and BC-1 ice-core samples had one and three unique T-RFs, respectively, but no unique T-RFs were identified in BC-T, which also only had five T-RFs in total. The number of T-RFs from the ice-core samples was much lower than in T-RFLP studies from non-cryospheric environments (Tiquia *et al.*, 2002; McGuinness *et al.*, 2006), but in the lower range of estimates from other cryospheric environments such as John Evans Glacier (Bhatia *et al.*, 2006).

The relative peak height of T-RFs can indicate their relative abundance within the bacterial communities. In the bacterial profiles analysed here, 88 per cent of T-RFs from the *Msp*I digestion had a relative peak abundance of

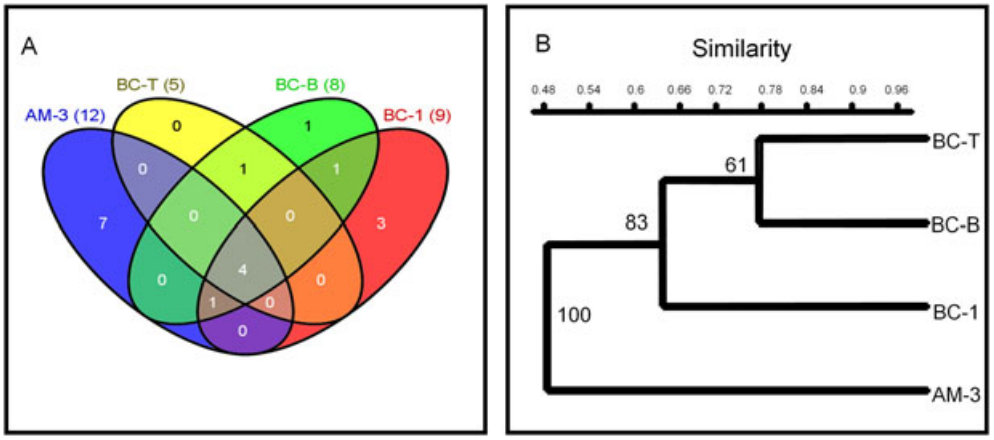

Figure 1 (A) Venn diagram illustrating the number of terminal-restriction fragments (T-RFs) per bacterial profile. (B) Bray-Curtis cluster analysis of 16S rRNA gene T-RF profiles from Amorphous Glacier (AM) and Boulder Clay (BC) obtained from 1000 bootstraps. This figure is available in colour online at wileyonlinelibrary.com/journal/ppp.

Table 2 Number and relative peak abundance of T-RFs with *Msp*I and *ScrF*I digestions detected in AM-3, BC-T, BC-B and BC-1 ice core samples.

T-RFs (bp)	Relative peak abundance (%)			
	AM-3	BC-T	BC-B	BC-1
*Msp*I digestion				
43	+ (1.3)			
48				+ (1.3)
73			+ (1.6)	+ (2.0)
81	+ (5.6)	+ (6.7)	+ (10.1)	+ (7.8)
145		+ (1.6)	+ (1.2)	
147				+ (1.2)
148	+ (1.7)			
149				+ (1.3)
279			+ (2.4)	
538	+ (1.3)			
553	+ (39.1)			
1205	+ (1.7)			
Sum	6	2	4	5
*ScrF*I digestion				
43	+ (3.1)		+ (1.3)	+ (1.6)
76	+ (1.2)	+ (1.3)	+ (1.2)	+ (1.6)
81	+ (15.6)	+ (16.2)	+ (32.5)	+ (32.4)
116	+ (1.3)			
145	+ (2.7)	+ (2.5)	+ (2.1)	+ (3.1)
796	+ (3.6)			
Sum	6	3	4	4
Total	12	5	8	9

T-RFs = Terminal-restriction fragments; AM = Amorphous Glacier; BC = Boulder Clay; bp = base pairs.

less than 10 per cent (Table 2). The greatest peak abundance was T-RF size 553 from AM-3 sample, with 39.1 per cent. In the *ScrF*I digestion, T-RF size 81 was the most abundant fragment, with a relative abundance of 32.5 per cent (BC-B and BC-1 samples), and 76 per cent of T-RFs had a relative peak abundance below 10 per cent. This could suggest that some taxa within the bacterial communities may dominate the overall abundance of the community profiles.

Bray-Curtis similarity cluster analysis (Figure 1B) suggested that BC T-RF profiles were similar to each other but clustered separately from the AM-3 ice-core sample, which varied lithologically and is of Holocene origin. Two possible explanations for these results are the brine pockets in Boulder Clay, with high salt concentrations created due to partially melted ice with hypersaline water intrusions, and the penetration of bacteria from the top to lower layers via liquid water or micro-channels in the ice. Because it was not possible with the current analysis and available data to confirm which factors contributed to the general differences in the bacterial profiles observed, additional analyses such as bacterial cell counts, cultivation studies, clone libraries and deep-sequencing community structure analysis are necessary to fully evaluate the diversity of the bacterial profiles established. Whole genome amplification could also be used to increase DNA concentrations recovered from the corers for downstream PCR analysis in any future analysis (Binga *et al.*, 2008). Such research will confirm the presence and viability of these psychrophilic bacterial phyla, and help confirm and correlate the community composition with the geological and habitat characteristics of Amorphous Glacier and Boulder Clay.

CONCLUSIONS

In conclusion, our molecular data confirmed, for the first time, the presence of bacterial DNA and suggested that distinct bacterial communities reside in the ice layers of Amorphous Glacier and Boulder Clay in Northern Victoria Land, Antarctica. It was not possible, however, with the current analysis and available data to determine which factors have led to the general differences in the bacterial profiles observed.

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