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

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

T-RFs were visualised as peaks in GeneScan

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 ScrFI 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

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