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Bacterial diversity and bioprospecting for cold-active enzymes from culturable bacteria associated with sediment from a melt water stream of Midtre Lovénbreen glacier, an Arctic glacier

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Abstract

Culturable bacterial diversity of Midtre Lovénbreen glacier, an Arctic glacier, was studied using 12 sediment samples collected from different points, along a transect, from the snout of Midtre Lovénbreen glacier up to the convergence point of the melt water stream with the sea. Bacterial abundance appeared to be closer to the convergence point of the glacial melt water stream with the sea than at the snout of the glacier. A total of 117 bacterial strains were isolated from the sediment samples. Based on 16S rRNA gene sequence analyses, the isolates ($n = 117$) could be categorised in to 32 groups, with each group representing a different taxa belonging to 4 phyla (*Actinobacteria*, *Bacilli*, *Flavobacteria* and *Proteobacteria*). Representatives of the 32 groups varied in their growth temperature range (4–37 °C), in their tolerance to NaCl (0.1–1 M NaCl) and in the growth pH range (2–13). Only 14 of 32 representative strains exhibited amylase, lipase and (or) protease activity and only one isolate (AsdM4-6) showed all three enzyme activities at 5 and 20 °C respectively. More than half of the isolates were pigmented. Fatty acid profile studies indicated that short-chain fatty acids, unsaturated fatty acids, branched fatty acids, cyclic and *cis* fatty acids are predominant in the psychrophilic bacteria.

Keywords: 16S rRNA gene; *Actinobacteria*; *Bacilli*; *Flavobacteria*; *Proteobacteria*; Extracellular enzyme activities; Phenotypic characteristics; Fatty acid profiles

1. Introduction

The Arctic region has several distinct habitats such as sea ice, glacial ice, permafrost, tundra wetlands, oceanic water, subglacial soil, periglacial soil, tundra soil, etc. Thus, the Arctic has served as a potential region for identifying novel psychrophilic bacteria with biotechnological potential. Furthermore, the Arctic has become a model system for global warming [38] and the Arctic fjords are regarded as key European sites for Arctic biodiversity monitoring [23].

Most earlier studies in the Arctic have focused on the abundance and biomass of bacteria in glacial fjords [13], glacial runoff [20], packed snow [2], subglacial and periglacial habitats [14], soil [23,37], Arctic sediment [28], permafrost [34], mountains [19] and other habitats. These studies indicated that the total bacterial number in these habitats varied depending on factors such as the nature of the habitat [13], the depth of the sample [28], temperature [2], the extent of vegetation [20], the level of nutrients [14], changes in the climate [37], etc. In addition, 16S rRNA gene clone libraries of Arctic tundra soils, subglacial samples and permafrost

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samples revealed many sequences that were related to the *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Firmicutes*, *Cytophaga–Flavobacteria–Bacteroides*, *Planctomyces* and *Gemmatimonadetes* [7,24,34].

Despite the fact that the Arctic has a number of glaciers, comparatively little is known about the bacterial diversity of the distinct habitats of the glaciers, such as glacial fjords [13], glacial runoff [20], subglacial and periglacial habitats [14], etc. In a recent study [20], bacterial production in glacial runoff and aquatic habitats along a 500 m transect from the ablation area of a Svalbard glacier (Midtre Lovénbreen, 78° 53′ N 12° 02′ E) down to a series of pro-glacial lakes in its forefield was monitored. It was observed that the bacterial abundance and production increased significantly along this transect and reached a maximum in the pro-glacial lakes. Furthermore, DGGE analysis indicated partial similarity between bacterial communities from the glacier runoff, while samples from the pro-glacial lakes did not display significant similarities [20]. However, attempts were not made to identify the various bacteria in the study. Furthermore, in these microorganisms, bioprospecting for cold-active enzymes might be important for biotechnology processes or medicine [9,10].

The present study is an attempt to determine bacterial abundance and viable bacterial diversity from the melt water stream of the Midtre Lovénbreen glacier, using sediment samples collected along a transect from the snout of Midtre Lovénbreen glacier up to the convergence point of the melt water stream with the sea. In addition, these microorganisms were also used for bioprospecting for pigments, fatty acids and cold-active enzymes.

2. Materials and methods

2.1. Sampling site

Midtre Lovénbreen glacier is located in the Kongsfjord region of Spitsbergen (78° 53′ 703″ N 12° 02′ 475″ E), Arctic. This glacier is about 6 km long, with an area of about 5.5 km^2 and a maximum thickness of about 180 m [4]. The altitude of the glacier at the snout is about 50 m and increases to about 600 m above sea level at the head wall. The distance from the snout of the glacier to the convergence point of the melt water stream with the sea is about 2–3 km.

2.2. Sampling method

Seven sediment samples (M1–M7) were collected on August 13, 2007, in sterile 50 ml Falcon tubes, all along the melt water stream of the glacier starting from the snout up until the convergence point (Fig. 1). The sediment was collected from the periphery of the stream where the depth was less than 50 cm. In addition, 5 more samples (MV–MZ) were also collected from small puddles adjacent to the above collection points (Table 1) (Fig. 1). The mean air temperature at the time of collection was 4.5 °C and the temperature of the sediment near the snout of the glacier was <1 °C; all along the transect it varied between 1 °C and 2 °C, and at the convergence point it was about 3 °C. In samples MV–MZ, the temperature was 4–5 °C. All samples after collection were stored at 5 °C in the Arctic. Subsequently, samples were transported in ice buckets, with ice packs which were pre-cooled to −70 °C. Direct contact between the ice packs and the samples was avoided. The samples reached the Centre for Cellular and Molecular Biology, Hyderabad, India, for further processing after about 24 h of air travel. At the time of opening, the temperature of the samples was about 8 °C.

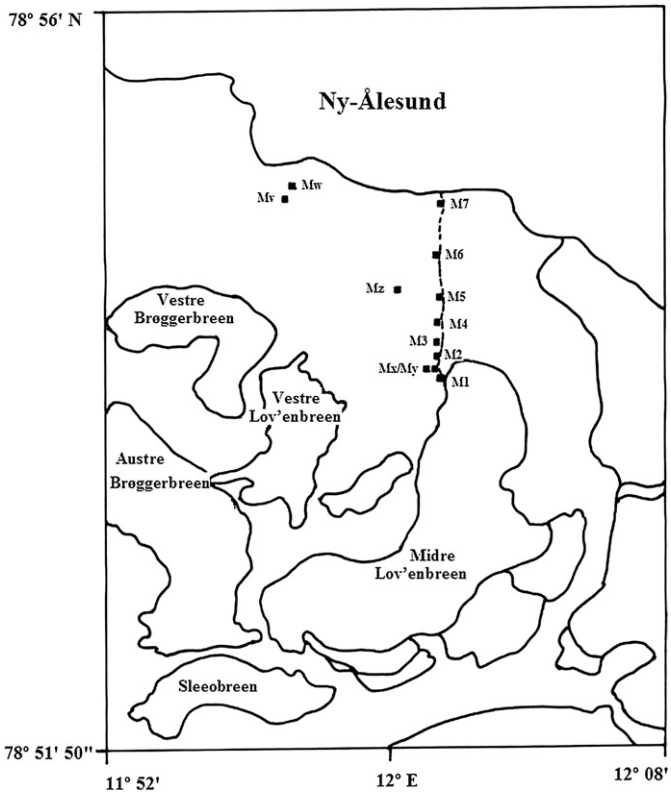

Fig. 1. Location of Midtre Lovénbreen glacier at Ny-Ålesund and sampling sites.

2.3. Isolation and culturing of bacteria

Approximately 100 mg of the sediment sample was suspended in 900 µl of sterile water and subjected to shaking for 2 h at 20 °C. The supernatant was serially diluted and 100 µl was plated on Antarctic Bacterial Medium (ABM) plates [peptone (0.5%, w/v), yeast extract (0.2%, w/v) and agar (2%, w/v)] and incubated at 10 °C for 15 days. Different morphotypes were purified and maintained on ABM plates. Total bacterial count in the sediment samples was determined by epifluorescence using the *Bac*Light™ Bacterial Viability Kit (Invitrogen, Oregon, USA) as per the instructions given in the kit. Bacteria were counted in a Petroff-Hausser counter using a fluorescent microscope (Axioplan 2, Zeiss, Germany).

2.4. Characterisation of the bacterial strains

Colony characteristics were observed with the help of a magnifying lens. Growth at different temperatures, pH and