



High diversity and distinctive community structure of bacteria on glaciers in China revealed by 454 pyrosequencing



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ABSTRACT

The bacterial diversity, community structure and preliminary microbial biogeographic pattern were assessed on glacier surfaces, including three northern glaciers (cold glaciers) and three southern glaciers (temperate glaciers) in China that experienced distinct climatic conditions. Pyrosequencing revealed that bacterial diversities were surprisingly high. With respect to operational taxonomic units (OTUs), *Proteobacteria* was the most dominant phylum on the glacier surfaces, especially *Betaproteobacteria*. Significant differences of the bacterial communities were observed between northern and southern glacier surfaces. The rare and abundant populations showed similar clustering patterns to the whole community. The analysis of the culturable bacterial compositions from four glaciers supported this conclusion. Redundancy analysis (RDA) and partial Mantel tests indicated that annual mean temperature, as well as geographical distance, was significantly correlated with the bacterial communities on the glaciers. It was inferred that bacterial communities on northern and southern glacier surfaces experienced different climate, water and nutrient patterns, and consequently evolved different lifestyles.

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Introduction

Glaciers represent extreme low temperature environments in the world, which cover more than 15 million square kilometres and occupy approximately 10% of the Earth's total land area. Most glaciers are located in the polar regions, and a few are located in mountainous areas above the snow line (<http://nsidc.org/cryosphere/glaciers>). The characteristics and types of glaciers are determined by the climate [31]. According to thermal characteristics, glaciers can be classified into three types: cold, temperate (warm) and polythermal. The temperate glaciers are at melting point throughout the year from the surface to the base [31]. However, cold glaciers are below their melting point from top to bottom, except in certain cases where the temperature of the surface layer can reach the melting point seasonally. This type of glacier only exists in regions with high latitude and high altitude where the mean annual temperature is extremely negative [27].

The cold and low nutrient concentrations make microbial communities on glaciers special and complex. The study of glacier microorganisms would yield valuable information concerning

microbial community composition, the mechanisms for cold-adaptation of microbial cells and the origin, evolution and limit of life on Earth. As the biodiversity of microorganisms in cold environments has been receiving more attention, plenty of knowledge of the bacterial communities on glaciers have been obtained based on both the use of culture-dependent and culture-independent approaches [1,29,32,33,37,38,43]. Amato et al. [1] summarised that bacteria from cold environments belonged to the *Proteobacteria* group (mainly *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*), *Cytophaga-Flavobacterium-Bacteroides* (CFB) and low G+C (LGC) and high G+C (HGC) Gram-positive bacteria. Several investigations of the microbial community have revealed some abundant genera, such as *Arthrobacter*, *Hymenobacter*, *Deinococcus*, *Cryobacterium*, *Flavobacterium*, *Polaromonas* and *Sphingomonas* [26,32,36]. However, because of methodological limitations, previous studies have reported mainly on community composition rather than structure. Few microbial investigations have employed 454 pyrosequencing of the 16S rRNA gene in order to obtain a snapshot of the microbial community structure on the surfaces of mountain glaciers. Currently, little biogeographic information concerning the biosphere on glacier surfaces is available [2,28], although Xiang et al. [44] found different bacterial community patterns between two glaciers on the Tibetan plateau. Therefore, further studies should be performed to understand better the biodiversity and bacterial biogeography on glacier surfaces.

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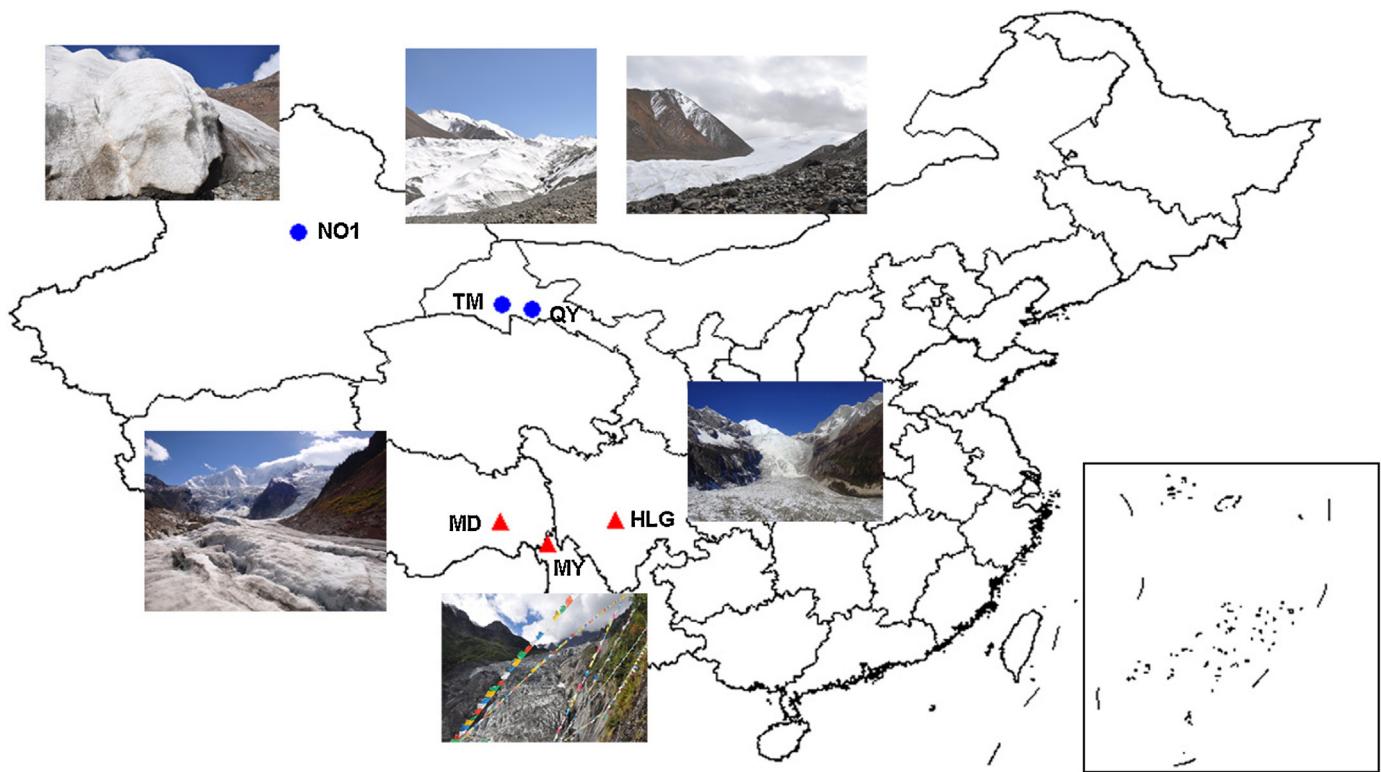


Fig. 1. The sampling sites in this study: NO1, TM and QY are located in northwest China; MD, HLG and MY are located in southwest China.

There are 48,571 glaciers [25] from southwest to northwest China, all of which are located in mountainous areas. Northwest glaciers are cold glaciers under the influence of a continental climate, and their constant temperature stratum achieves negative temperatures. However, southwest glaciers are temperate glaciers under the influence of a maritime climate, and their constant temperature stratum is at the pressure melting point [18]. The glaciers in these two geographic regions enabled us to characterise the bacteria compositions and compare their biogeographic distributions under these different climates.

In this context, 454 pyrosequencing of the 16S rRNA gene was employed to gain an in-depth insight at a greater resolution into the biodiversity of bacterial communities on the surfaces of both northwest and southwest glaciers. From the biodiversity surveys and microbial biogeographic comparisons, we tried to evaluate the influences of environmental (physicochemical and climatic) variables and geographic characteristics on the biodiversity, as well as, finally, to discover a possible preliminary microbial biogeographic pattern on the glacier surfaces.

Materials and methods

Sample collection and description of sampling sites

Six glaciers were selected for sample collection: Xinjiang No. 1 Glacier (NO1), Qiyi Glacier (QY), Toumingmengke Glacier (TM), Hailuoguo Glacier (HLG), Mingyong Glacier (MY) and Midui Glacier (MD) (Fig. 1, Table 1). NO1, QY and TM are located in northwest China, whereas HLG, MY and MD are located in southwest China. Surface ice from all six glaciers was collected from the glacier tongues in September 2011 using three replicates that were 100 m apart. The samples that harboured surface dusts were chipped to approximately 0–20 cm thick by a sterile hatchet. Sterile sample bottles were used to transport the ice samples in an insulated container with dry ice and they were then stored at -80°C in

the laboratory. Ice samples were melted at ambient temperature before filtration. Three replicates of each sample were mixed and filtered together onto sterile cellulose acetate membranes (pore size $0.22\ \mu\text{m}$; Millipore) using the pump and filtration device for DNA extraction and the determination of physicochemical compositions. For avoiding cross-contamination, the filtration unit was rinsed three times with sterile Veolia water before each sample filtration.

HLG is situated on Gongga Mountain, which is in the eastern Hengduan Mountain Range in Sichuan Province. MY is situated on Meri Snow Mountain, which is in the western Hengduan Mountain Range in Yunnan Province. MD is located at the juncture of the Nyenchen Tanglha Mountain Range with the Boshula Mountain chain (the far western chain of the Hengduan Mountain Range) in Tibet. The Hengduan Mountain Range occupies the southeastern part of the Qinghai–Tibet Plateau. As the only mountain range in China under the influence of a high-altitude westerly circulation and the monsoon circulation of the Indian and Pacific oceans, Hengduan Mountain Range has a favourable maritime climate, with very obvious wet and dry seasons. NO1 sits on the Tianshan Mountain Range, which is a large mountain range in Central Asia, running through central Xinjiang, China, with the west end stretching into Kazakhstan. In the area of the Tianshan Mountain Range, there is an obvious continental climate, with distinct cold and warm seasons during the year. TM and QY are in the territory of Gansu province

Table 1
Geographic characteristics of the glaciers from northwest and southwest China.

Region	Glacier	Latitude	Longitude	Altitude (m; a.s.l.)
Northwest China	NO1	43.12 N	86.81 E	3,838
	QY	39.25 N	97.75 E	4,041
	TM	39.50 N	96.52 E	4,278
Southwest China	HLG	29.56 N	101.97 E	3,457
	MD	29.45 N	96.50 E	3,901
	MY	28.45 N	98.76 E	2,970

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