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Bioprospecting a glacial river in Iceland for bacterial biopolymer degraders

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ABSTRACT

The glacial river Jökulsá á Fjöllum, which originates in the Vatnajökull ice cap and flows through a large basaltic tephra desert on its way to discharge into the Arctic Ocean, presents a number of unique microbial habitats heretofore unexplored. We sampled river water, sediment and selected other biotopes at 12 sampling points along the river from source to mouth and generated a collection of 382 purified and confirmed reculturable psychrotrophic bacterial strains. Partial 16S rDNA sequencing yielded 19 genera and 4 non-genus specific assignments in 4 bacterial phyla, with pseudomonads and flavobacteria being particularly well represented. A large portion of the isolates produced extracellular enzymes at 15 °C, including amylase, betaglucanase, cellulase, protease and laccase.

1. Introduction

Jökulsá á Fjöllum (JáF) is among the largest glacial rivers in Iceland. It originates in the Dyngjujökull, Brúarjökull and Kverkfjöll areas of the Vatnajökull ice cap and flows north for 206 km to discharge to the sea in Öxarfjörður bay (Fig. 1). It is located in a volcanically active area and, thus it flows for most of its length through recent basalt formations. JáF is highly loaded with suspended solids, discharging about 8×10^6 metric tons of solids per annum (Tómasson et al., 1996). Suspended solids are an important habitat for bacteria in flowing water, presenting a solid surface for attachment (Logan and Hunt, 1987). In the highland region, the JáF riverbed is shallow and covered by a thick layer of sandy sediment, but as the river exits the highlands it flows for 30 km through a deep canyon until it fans out on the Öxarfjörður alluvial cone. While JáF is characterized as a glacial river and is to a large extent glacier-fed, it also contains significant amounts of spring water. The river water is modestly alkaline, with typical pH values in the 7.4 to 8.1 range (Óskarsdóttir, 2007). Dissolved electrolyte content is comparatively high, with typical conductivity values of river water about 100 to 150 μS cm^{-1} (Óskarsdóttir, 2007). The average flow rate is about 200 m^3/s (Sigurðsson, 1990). Conductivity, flow rate and water table height are continuously monitored by the Icelandic Meteorolical Office at three points in JáF and the large eastern tributary Kreppa (http://en.vedur.is/#tab=vatnafar).

Among likely sources of bacteria in the river are glacial ice, highland desert soil and soil from vegetated oases such as Bæjarlönd and Herðubreiðarlindir. Human habitation is very scarce in the JáF watershed and almost exclusively restricted to the alluvial cone. Significant human impact on the microbiota is therefore highly unlikely, particularly in the highland region. The microbiota of a flowing river reflects the microbial communities in the river's watershed as microbes from surrounding biotopes are washed into the river by riverbed erosion, groundwater, snowmelt, glacial melt, rainwater and winds (Atlas and Bartha, 1993). A large part of the river microbiota is thus of allochthonous origin. Nevertheless, a significant part of the river microbiota, regardless of its origin, is not simply carried along in a passive state but rather contribute to the chemical processes occurring within the river water. Bacterial metabolic activity can therefore be considerable and is generally the driving force of organic carbon solubilization and biopolymer degradation (del Giorgio and Davis, 2003; Guo et al., 2007). Among the most commonly observed phyla and classes of bacteria in river water are the *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Verrucomicrobia* and *Planctomycetes* (Crump et al., 2009; Logue et al., 2008; Methe et al., 1998; Vallieres et al., 2008; Zwart et al., 2002). Crump et al. found that the composition of the microbiotas of the six largest river systems in the Arctic followed predictable seasonal patterns (Crump et al., 2009).

Although the bacterial biota of glacial ice has received considerable attention in the last several years (Loveland-Curtze et al., 2009; Pradhan et al., 2010; Reigstad et al., 2008; Simon et al., 2009; Zhang et al., 2008), the supraglacial and englacial microbiotas of the Vatnajökull ice cap have not been studied to date. Subglacial-lake and tephra microbial communities have, however, been investigated

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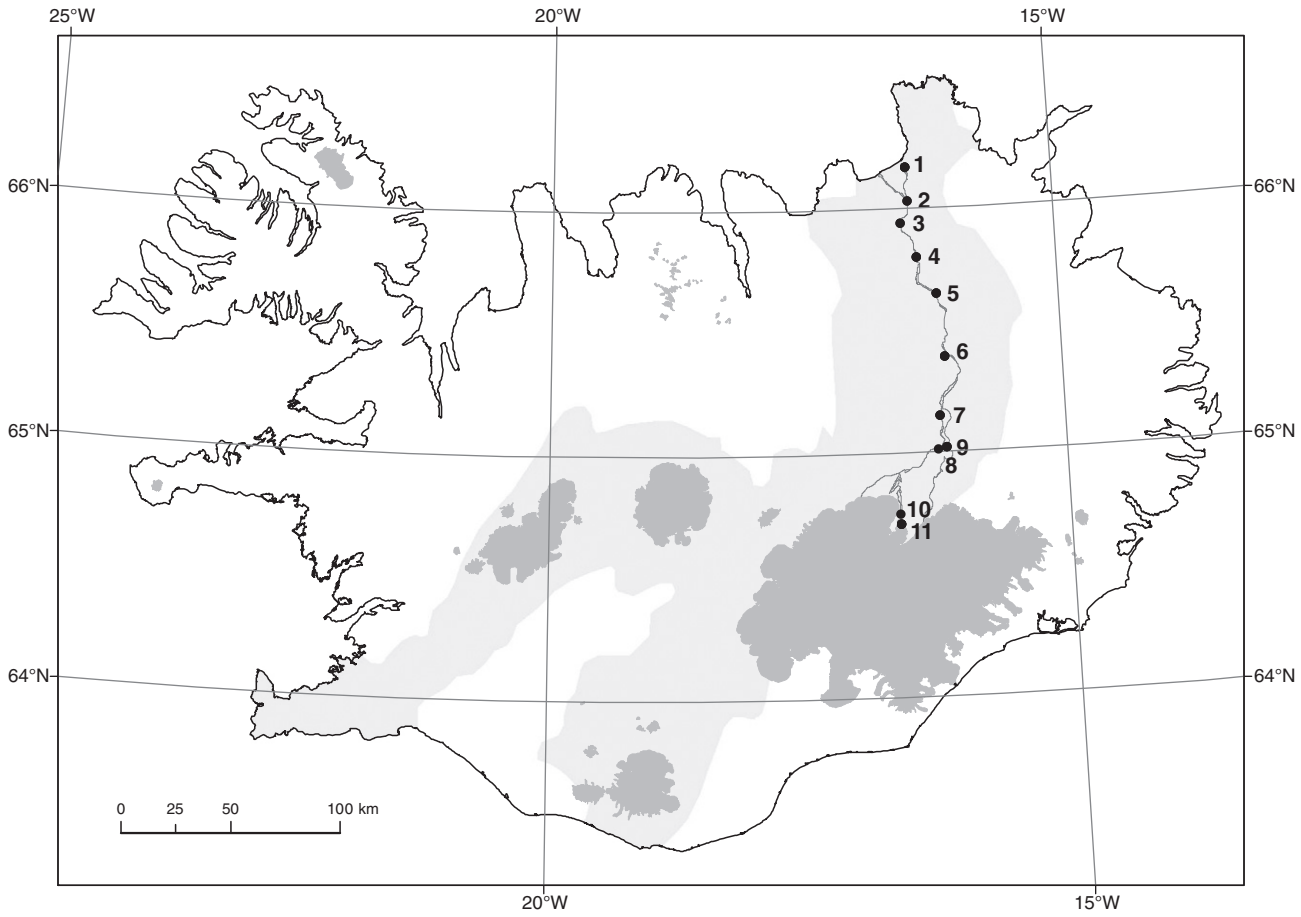

Fig. 1. The river Jökulsá á Fjöllum is located in NE Iceland, originating in the northern part of Vatnajökull ice cap. Major glaciers in Iceland are shaded dark grey, the main Volcanic Rift Zone is shaded light grey. Sampling locations along the river are indicated by dots and numbered as in Table 1.

in the Grímsvötn and Skaftárkatlar areas in the western and southern parts of the ice cap (Gaidos et al., 2004, 2009; Marteinsson et al., 2013). These studies revealed on the one hand diverse, Proteobacteria-dominated communities of psychrotolerant bacteria in the Grímsvötn area (Gaidos et al., 2004), and on the other a strongly oligarchic chemotrophic community dominated by *Acetobacterium*, *Thermus* and *Paludibacter* in the Skaftárkatlar area (Gaidos et al., 2009). Comparing these studies illustrates the somewhat conflicting effects of glacial and geothermal effects on these habitats, making Vatnajökull, which straddles Iceland's main volcanic rift zone (Fig. 1), and its rivers particularly intriguing from a microbial ecology perspective.

The JáF microbiota has not been investigated before to our knowledge, but recently the microbiota of several habitats along Glerá, a smaller semiglacial river in northern Iceland, was investigated (Markúsdóttir et al., 2012). The culturable microbiota of the pristine, upper part of that river was found to be characterized by protein-degrading pseudomonads similar to members of the *Pseudomonas fluorescens* species group, with members of the Bacilli, Actinobacteria, Alphaproteobacteria and Sphingobacteria also present.

The main objective of the present study was to bioprospect this cold and barren environment for bacteria of biotechnological interest, specifically those displaying extracellular enzymatic activity. The main objectives of the present study were to (1) assess the diversity of the culturable bacterial microbiota of JáF and its immediate surroundings, (2) establish a culture collection of psychrotrophic JáF isolates, and (3) screen the culture collection for production of cold-active enzymes, including laccase, amylase, beta-glucanase, cellulase and protease.

2. Methods

2.1. Sampling and physicochemical measurements

Samples of river water and riverbed sediment were collected from eleven sampling sites along Jökulsá á Fjöllum river during June and August 2011 (Fig. 1, Table 1). River water samples were collected in triplicate in sterile 500-ml plastic bottles and were obtained from circa 20 cm under the surface of rapidly flowing river water. During sampling, water temperature was measured with a hand-held thermometer. Riverbed sediment and soil samples were collected with sterile spatulas into sterile 50-ml plastic centrifuge tubes. Exposed riverbed samples (JF16, JF37 and JF44) were taken from the riverbank at a distance of less than 2 m from flowing water and a depth of approximately 5 cm below the surface. Samples were transported on ice to the laboratory at Akureyri where all further processing was carried out. Electrical conductivity in thawed river water and glacial meltwater samples was measured at 21 °C with a CON 510 series conductivity meter (Oakton Instruments, Vernon Hills, IL, USA) calibrated against 84 and 1140 μS cm^{-1} KCl standards; pH was measured at 21 °C with an Orion Dual Star benchtop pH meter (Thermo Fisher Scientific, Waltham, MA, USA) fitted with a Ross pH electrode and calibrated against standard buffers in the pH 4.0 to 10.0 range.

2.2. Media, culture conditions and strain isolation

Water samples were plated in duplicate directly onto R2A (Becton Dickinson, Franklin Lakes, NJ, USA) and PCA (Becton Dickinson), 0.1