Cultured Bacterial Diversity and Human Impact on Alpine Glacier Cryoconite

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The anthropogenic effect on the microbial communities in alpine glacier cryoconites was investigated by cultivation and physiological characterization of bacteria from six cryoconite samples taken at sites with different amounts of human impact. Two hundred and forty seven bacterial isolates were included in Actinobacteria (9%, particularly Arthrobacter), Bacteroidetes (14%, particularly Olella), Firmicutes (0.8%), Alphaproteobacteria (2%), Betaproteobacteria (16%, particularly Janthinobacterium), and Gammaproteobacteria (59%, particularly Pseudomonas). Among them, isolates of Arthrobacter were detected only in samples from sites with no human impact, while isolates affiliated with Enterobacteriaceae were detected only in samples from sites with strong human impact. Bacterial isolates included in Actinobacteria and Bacteroidetes were frequently isolated from pristine sites and showed low maximum growth temperature and enzyme secretion. Bacterial isolates included in Gammaproteobacteria were more frequently isolated from sites with stronger human impact and showed high maximum growth temperature and enzyme secretion. Ecotypic differences were not evident among isolates of Janthinobacterium lividum, Pseudomonas fluorescens, and Pseudomonas veronii, which were frequently isolated from sites with different degrees of anthropogenic effect.

Keywords: bacterial diversity, cryoconite, human impact

Cryoconite holes are vertical cylindrical melt holes in the glacier surface, which are produced when glacier surfaces with deposition of wind-borne debris are heated by solar radiation (Wharton et al., 1985). The debris in the cryoconite hole is a mixture of inorganic and organic particulates. Most of the organic carbons contained in the cryoconite holes originate allochthonously rather than by autochthonous primary production by autotrophotrophic bacteria or algae (Stibal et al., 2008). Cryoconite holes are regarded to be an important glacier habitat for microbial life, as sediments from cryoconite holes are characterized by lower pH values, finer texture, higher water content, and higher concentration of nutrients than samples from supraglacial moraines and kames, and support higher levels of microbial life (Stibal et al., 2006). Cryoconite holes have been known to support active microbial communities including bacteria, microalgae, fungi, and metazoans (Mueller et al., 2001; Takeuchi et al., 2001; Margesin et al., 2002; Säwström et al., 2002; Christner et al., 2003; Stibal et al., 2006). Maintaining a very low temperature with ice at the bottom, cryoconite holes are habitats harboring cold-adapted microbial life and cold-active enzymes (Margesin et al., 2002, 2003b, 2005, 2007; Christner et al., 2003).

With the advancement of molecular techniques, cultivation-independent approaches for describing microbial diversity have opened up new perspectives for microbial ecology and have been widely used in research on microbial communities (Bai et al., 2006). Although cultivation-independent approaches avoid the limitations of traditional culture-based methods, by which approximately 1% of the environmental bacteria can be cultured by general laboratory practices (Kirk et al., 2004; Bai et al., 2006), the culture-based approach applied in this study has benefits over molecular approaches in some respects. It is not possible to determine and compare the physiological characteristics of samples using a molecular approach. Further, the characterization of culturable microorganisms can provide information on the ecological roles of at least some members of the microbial community, and thus may augment knowledge of community structure derived from direct molecular approaches (Jiang et al., 2006). In addition, a culture-based approach is required to assemble a collection of microorganisms on which to conduct biochemical, genetic and physiological experiments, and within which to probe inter- and intra-species interactions (Jiang et al., 2006).

Microbial communities are highly sensitive to environmental changes and respond rapidly to changing environmental conditions or anthropogenic stress (Webster and Negri, 2006). Although a number of studies on the relationship between the microbial community composition and human impacts or environmental gradients have been reported for various natural environments, including a stream-groundwater exchange zone, soils, caves, sediments, fresh water, coastal water, and biofilms (Øvreås et al., 1998; Paerl, 1998; Dorigo et al., 2002; Hancock, 2002; Margesin et al., 2003a; Powell et al., 2003; Nocker et al., 2004; Webster and Negri, 2006; Ikner et al., 2007), studies on the influence of human impact on microbial community composition of cryoconites have not been reported. In this study, the influence of human impact on the microbial com-

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munities of cryoconite holes from an alpine area was investigated by comparing diversity and physiological characteristics of cultured bacterial isolates from samples of six cryoconite holes with different frequencies of human visits. Growth temperature and hydrolytic enzyme secretion of microorganisms were studied based on the assumption that frequent human visits may increase the opportunity to introduce microorganisms of human origin and to enrich populations of microorganisms utilizing exogenous proteins and lipids.

Materials and Methods

Study site and sample collection
Cryoconite samples were collected from mountains in the Austrian Alps during June and August 2006 and transported to the laboratory at the University of Innsbruck as quickly as possible. Samples (0.9 ml) were placed into cryo-vials with 0.9 ml of 20% glycerol and transported to the laboratory at the Korea Polar Research Institute in an ice-box packed with dry-ice. They were preserved at -80°C until used. The degree of human impact on the sampling site was designated as 0 (none), 1 (weak), 2 (strong), and 3 (very strong) according to the frequency of human visits to the area of the sampling site. The altitude ranged between 2200 and 2900 m above sea level and the parent rock of the glacier was mostly silicate except for one sample with carbonate rock (Table 1).

Bacterial cultures

For cultivation of bacterial isolates, samples were serially diluted up to 10^{-7} in sterilized distilled water and 100 μl of the diluted sample suspension were spread on five kinds of medium: nutrient agar (Difco), 1% tributyrate (Sigma, USA), respectively. The plates were incubated for 3 days for protease secretion, and for 7 days for lipase secretion at 10°C and 20°C. Enzyme secretion was scored as follows: 0, no clear zone; 1, faint clear zone; 2, clear zone was evident and width of clear zone was smaller than the radius of the colony; 3, width of clear zone was bigger than the radius and smaller than the diameter of the colony, 4, width of clear zone was bigger than the diameter of the colony.

Phylogenetic analysis of bacterial isolates

Bacterial strains were identified by sequence similarity and phylogenetic analysis of 16S rRNA gene sequences. The 16S rRNA gene was amplified from a single colony of pure cultures with two universal primers, 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-GTT TAC CTT GTC TTA CTC AG-3′), as described by Lane (1991). PCR was carried out with 25 μl reaction mixtures containing 1× PCR reaction buffer, 200 μM of dNTPs, 0.2 μM of each primer, a single colony as a template and 1 unit of Taq DNA polymerase (In-Sung Science, Korea). The PCR procedure included an initial denaturing step at 95°C for 5 min and 30 cycles of amplification (95°C for 30 sec, 56°C for 30 sec, and 72°C for 30 sec) and a final extension step at 72°C for 5 min. PCR products were purified using the AccuPrep PCR Purification kit (Bioneer, Korea) and sequenced with the same primers used for PCR amplification. The sequence of the 16S rRNA gene was compared with that of type strains available in the EzTaxon database (Chun et al., 2007) to find closely related species and choose reference sequences for phylogenetic analyses. Phylogenetic trees were reconstructed by the neighbor-joining method (Saitou and Nei, 1987) based on the distance matrix generated according to the Kimura’s two-parameter model (Kimura, 1980) using PHYLIP ver. 3.69 (Felsenstein, 2009). The confidence level of the tree topology was evaluated by bootstrap analysis using 1,000 sequence replications. Species affiliation of a bacterial isolate was determined when the isolate formed a monophyletic group with reference species and had 99% or higher similarity. Sequences were submitted to NCBI GenBank under the accession numbers HQ624836-HQ625082.

Results

Bacterial cultures and physiological characteristics

Two hundred and forty seven bacterial isolates were obtained from samples taken at six cryoconite sites with different levels of human impact. Most of the isolates could grow between...