Bacterial microbiota associated with *Umbilicaria* spp. lichens in continental and maritime Antarctic, Arctic and Alpine regions

Zichen He¹, Takeshi Naganuma¹, Ryosuke Nakai², Megumu Tsujimoto³, Hiroshi Kanda⁴, Satoshi Imura⁴, Jun Uetake⁵, Martin W. Hahn⁶, and Peter Convey⁷

¹ Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-hiroshima, 739-8528, Japan

 2 Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology, Sapporo, Japan

³ Faculty of Environment and Information Studies, Keio University, Fujisawa, 252-0882, Japan

⁴ Bioscience Group, National Institute of Polar Research, Tachikawa, 190-8518, Japan

⁵ Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, 060-0811, Japan

⁶ Research Department for Limnology, University of Innsbruck, Mondsee, A-5310, Austria

⁷ British Antarctic Survey, High Cross, Madingley Road, Cambridge, CB3 0ET, UK

Lichens are common and widely distributed symbiotic organisms. Increased research attention is being given to the bacterial diversity associated with lichens. This study aimed to analyze the bacterial diversity and their physiological or ecological roles in the lichen symbiosis using culture-independent phylogenetic analyses.

A total of 44 lichen samples were obtained from continental and maritime Antarctic, Arctic and Alpine regions. In the two Antarctic regions, 18 samples were obtained from the continental Antarctic (Syowa station) and four samples from the maritime Antarctic (Signy Island); in the Arctic region, two samples were obtained from Kugluktu (Canada), two from Gamvik (Norway), and five from Enotekio and Levi (Finland); in the Alpine region, five samples were obtained from the Eastern Alps (Austria) and 11 from the Rwenzori Mountains (Uganda). All the sampled lichen-forming fungi were confirmed to be affiliated with the ascomycete genus *Umbilicaria* through Sanger sequencing.

Lichen-associated bacterial diversity was assessed using Illumina MiSeq targeting the V3-V4 region of the bacterial 16S rRNA gene. A total of 2,038,816 reads were obtained for analysis. Phylotypes (OTUs) were classified using a 97% similarity cutoff. 1,028,426 reads were obtained from Antarctic samples, 439,303 from Arctic samples and 571,087 from Alpine samples. In Antarctic samples, the average number of reads per sample was 46,747 representing an average of 478 OTUs; in Arctic samples, these values were 48,811 and 254, and in Alpine samples 35,693 and 426. Bacterial phylum composition identified from samples from Syowa Station was distinctly different from the other sampling regions. The dominant phylum in the 18 Syowa samples was *Bacteroidota* (66.46%), while in the 26 samples from other regions (including Antarctic Signy Island) it was *Pseudomonadota* (51.65%). This difference was confirmed using Principal Component Analysis. The assigned OTUs included four cosmopolitan taxa at species rank. These four OTUs affiliated with *Gluconacetobacter* or new genera of the *Acetobacteraceae* family, which is known to include nitrogen-fixing members.



Figure 1. Lichen sampling locations in Arctic (green), Alpine (blue) and Antarctic (red) regions, indicating the number of samples obtained at each location.