Analysis of bacterial microbiota associated with genus *Umbilicaria* lichen by Illumina MiSeq and Sanger sequencing methods

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Symbiosis in its simple definition describes close and long-term relationships between different species. In the nineteen centuries when biologists discovered the co-existing of fungi and algae generating biological structures known as lichen thallus, they coined the term 'symbiosis' to describe the nature of lichen. The lichen symbiosis of dual partnership between mycobiont and photobiont had recently been challenged and broadened. Few studies proposed lichens to be recognized as a 'multi-species symbioses' rather than dual, or tripartite. Those studies found diverse species of fungi besides the mycobiont, different species of algae co-existing with the photobiont, and a diverse bacterial microbiota. Of all the multi-species associated with lichen, only the mycobiont and photobiont have functional roles crucial for the development of lichen thallus. While the role of bacterial microbiota associated with lichen remained unknown, recent metagenomics and meta-proteomics provided strong evidence of bacterial involvement in nutrient provision, degradation of older thallus parts, nitrogen-fixing, detoxification as well as protection against biotic and abiotic stresses for the lichen.

Metagenomic studies largely contributed in identifying bacterial microbiota associated with lichens; however, bacterial compositional differences within the same lichen did occur, and various reasons were attributed to this phenomenon. Two of the major reasons are the sequencing methods employed and the databases used. As such, this study compared 'Sanger' and 'Illumina MiSeq' sequencing methods which utilized NCBI nr and EzTaxon-e databases. Since little is known on the bacterial microbiota associated with genus Umbilicaria lichens, this study used a total of 13 rock-inhabiting Umbilicaria specimens from four continents of North America, Europe, Africa and Antarctica.

In East Antarctica, 5 specimens were collected along the coast of Lützow-Holm Bay. Another 5 specimens were collected within an alpine zone of the Rwenzori Mountains in Uganda, and 1 specimen was obtained from northern Finland. Two specimens preserved at the herbarium of National Institute of Polar Research in Tokyo were originally collected from Nunavut, in north Canada. DNA were extracted, then 'Sanger' and 'Illumina MiSeq' sequencings were employed. 'Sanger' sequencing produced 1,500 bp of 16S rRNA gene, and 'Illumina MiSeq' sequencing produced 446 bp on v3 and v4 regions of 16S rRNA gene. At 97% similarity cut-off, sequences of 'Sanger' sequencing were BLASTN searched against NCBI nr database. Similarly, sequences of 'Illumina MiSeq' were BLASTN searched against EzTaxon-e database. Utilizing packages from R, calculations for rarefactions, hierarchical clustering, heatmaps and beta-diversity were done.

Profiling of bacterial microbiota using 'Sanger' and 'Illumina MiSeq' sequencings showed a degree of similarity. Although the 'Sanger' sequencing constituted a limited volume of data compared to 'Illumina MiSeq', it obtained 5 dominant phyla (Proteobacteria, Acidobacteria, Bacteroidetes, and Planctomycetes as well as plastid-derived Chlorophyta) that were also detected by 'Illumina MiSeq' sequencing. The massive sequences by 'Illumina MiSeq' sequencing obtained more bacterial taxa. In both methods, Antarctic samples differ in composition due to abundant Bacteroidetes that constituted predominant *Mucilaginibacter* and *Hymenobacter*. Other samples had high abundance of Acidobacteria (*Edapobacter, Granulicella* and *Terriglobus*) detected by 'Sanger' sequencing, which were not as dominant as Proteobacteria that was detected by 'Illumina MiSeq' sequencing. Interestingly, Antarctic samples composed of abundant algal family Oocystaceae, and samples of Finland and Uganda have dominant algal family Chlorellaceae as observed in 'Illumina MiSeq' sequencing. Plastid-derived algae was less detected in Canada samples for both sequencing methods, and this may due to treatments prior to preservation in herbarium.