Bacterial community structures of environmental sample and enrichment cultures of the hyper-saline lake Zakuro in the Langhovde, East Antarctica.

Kaori Iiyama[1], Hanae Seki[2], Akinori Kawamata[3], Satoshi Imura[4, 5], Hiroyuki D. Sakai[2] and Norio Kurosawa[1, 2]

[1]*Graduate School of Science and Engineering, Soka University*

[2]*Faculty of Science and Engineering, Soka University*

[3]*Ehime Prefectural Science Museum*

[4]*National Institute of Polar Research*

[5]*The Graduate University for Advanced Studies (SOKENDAI)*

Antarctica has the greatest diversity of lake types on Earth (Laybourn-Parry & Pearce, 2016; Pickard et al., 1986). Indeed, many lakes, consisting of various salinity, area, and depth, are located in Antarctica. These lakes have been considered low primary production because of the low temperature and significant periods of ice formation. Therefore, (micro)organisms in these lakes were generally considered oligotrophic with low biodiversity. However, decades of research have revealed that considerably diverse prokaryotes exist in those lakes (Vincent et al., 2008; Kurosawa et al., 2010; Laybourn-Parry & Pearce, 2016; Chaya et al., 2019). Lake Zakuro is a marine relic lake located in the Langhovde, East Antarctica, which possesses unique physicochemical parameters (i.e., hyper-saline, oligotrophic, and low temperature) that are not observed in temperature zones. Therefore, Bacteria in Lake Zakuro probably have unique physiology and ecology to adapt to this harsh environment. This study examined the bacterial community structures of environmental sample and enrichment cultures derived from Lake Zakuro by 16S rRNA gene amplicon sequence analyses.

Lake water sample, including the bottom mud, was collected on January 22, 2013. At that time, the water temperature and pH were 9.9℃ and 8.0, respectively. To reveal the bacterial community structure in Lake Zakuro, DNA was directly extracted from the sample and used for the 16S rRNA amplicon sequence analysis. The representative sequences were then applied to the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information for species identification. To find out what species of bacteria detected in the lake sample could be cultured, enrichment cultivations were conducted. The lake water sample was inoculated into the enrichment medium (pH 8.0) consisting of artificial seawater (salinity 80‰), 1xMBS (Kurosawa et al., 1998), and yeast extract (0.1%). Incubation temperatures were set at 5, 10, 15, 20, and 25℃. After reaching the stationary phase, the cultures were centrifuged, and the enriched microbial DNAs were extracted, followed by the 16S rRNA amplicon sequence analyses.

The bacterial Community in Lake Zakuro consisted of 19 phyla and was further classified into 179 genera. The most frequently detected phylum was Proteobacteria (39% of total sequences), in which α-Proteobacteria was mostly dominant (66% in the phylum), followed by γ-Proteobacteria (33%). Oligoflexia, β- and δ-Proteobacteria were also detected as minor components (<1%). This proteobacterial composition has the characteristics of marine bacteria. Other than Proteobacteria, phyla Bacteroidetes (19%), Verrucomicrobia (14%), Cyanobacteria (14%), Planctomycetes (2.0%), Actinobacteria (1.7%), and Campylobacterota (1.2%) were also detected. In addition, four phyla belonging to the Candidate Phyla Radiation (CPR) were detected at 1.4%. The CPR is a supergroup of uncultured enigmatic species that desired to be cultured and characterized in detail. As a result of enrichment cultivation, the growth of microorganisms was confirmed in the cultures at all temperatures. Based on the BLAST analysis, bacterial sequences in all the cultures were classified into four phyla, 30 genera, and 57 species. Thirty of the 57 species showed less than 98.2% homology to the described species, suggesting that these species are novel bacteria. However, their detection frequencies were at a maximum of 1.8%. It may be difficult to separate them from the enrichment cultures by the extinction dilution method. As mentioned above, 179 genera were detected in the environmental sample, but only 30 were detected in the enrichment cultures. In this enrichment, we used a single culture medium under aerobic conditions and changed only the temperature. To enrich the novel bacteria detected in Lake Zakuro to a much higher percentage, it is necessary to perform enrichment cultivation under more conditions with various media types.

References

Chaya, A., Kurosawa, N., Kawamata, A., Kosugi, M. and Imura, S., Community structures of bacteria, archaea, and eukaryotic microbes in the freshwater glacier lake Yukidori-Ike in Langhovde, East Antarctica. Diversity, 11(7), 105, 2019.

Kurosawa, N., Itoh, Y. H., Iwai, T., Sugai, A., Uda, I., Kimura, N., Horiuchi, T. and Itoh, T. *Sulfurisphaera ohwakuensis* gen. nov., sp. nov., a novel extremely thermophilic acidophile of the order Sulfolobales. Int. J. Syst. Bacteriol., 48(2), 451, 1998.

Kurosawa, N., Sato, S., Kawarabayasi, Y., Imura, S. and Naganuma, T., Archaeal and bacterial community structures in the anoxic sediment of Antarctic meromictic lake Nurume-Ike. Polar Science, 4(2), 421-429, 2010.

Laybourn-Parry, J. and Pearce, D., Heterotrophic bacteria in Antarctic lacustrine and glacial environments. Polar Biology, 39(12), 2207-2225, 2016.

Pickard, J., Adamson, D. A. and Heath, C. W., The evolution of Watts Lake, Vestfold Hills, East Antarctica, from marine inlet to freshwater lake. Palaeogeography, Palaeoclimatology, Palaeoecology, 53(2-4), 271-288, 1986.

Vincent, WF. and Laybourn-Parry (Eds.), J., Polar lakes and rivers: limnology of Arctic and Antarctic aquatic ecosystems. Oxford university press, 79, 2008.