December 2018
No.2018-010-e

Journal of Integrated Creative Studies

Report on my visit at the Center for Ecological Researches (CER) of Kyoto University in 2018

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Abstract. This report summarizes my visit to the Center for Ecological Researches (CER) of Kyoto University in 2018, supported by the International Research Unit of Advanced Future Studies. The report presents the description of my one- month activity at CER, and gives a brief research progress on discovery of neurotoxic saxitoxin producing cyanobacterium *Lyngbya wollei* (Farlow ex Gomont) Speziale and Dyck in Lake Biwa. This is the first report for the toxic *Lyngbya wollei* in Asia.
Key words: Benthic, Biwa**,** Cyanobacteria, *Lyngbya wollei*, Saxitoxin

1. General description of my visit at CER

From August 1, 2018 to August 31, 2018, I was working at the Center for Ecological Researches (CER) as a visiting professor, supported by the International Research Unit of Advanced Future Studies. I am so proud that this is the second time for me to obtain this professorship. I appreciate again the financial support from the International Research Unit of Advanced Future Studies and invitation from Prof. Nakano, the director of CER.

During my stay at CER this time, I was not able to take the boat for the field survey on Lake Biwa since the boat "Hasu" was seriously damaged. Alternatively, I was driving to Kitayamada, the south port of Lake Biwa, for collecting bloom samples since the first occurrence of cyanobacterial bloom in 2018 was reported there on August 6. We collected samples for two times, on August 12 and August 28, respectively. After the survey, we observed the samples and made the identification of dominant cyanobacterial species under the light microscope. Meanwhile, DNAs from the samples were also extracted for further analyses. During August 22-23, I had a trip to Tokyo for visiting Tokyo University of Agriculture and Technology and University of Tokyo.

2. Main activities

2.1 Presentations

I gave two talks during my visit to the two universities. One was hosted by Prof. Hosomi in Environmental Engineering, Tokyo University of Agriculture and Technology on August 22nd, as titled as "Molecular diversity and detection on cyanobacterial blooms and cyanotoxins in China waters;

another one was invited by Prof. Nakajima in Environmental Engineering, University of Tokyo on August 23rd, to present on "Cyanobacterial blooms in China: diversity, distribution and treatment".

2.2 Preparation of Joint proposal

Using this opportunity of my visit to Japan, Prof. Nakano at CER initially proposed the idea for submitting an international research proposal by three Asian countries including Japan, China and Indonesia through JSPS core -to-core, and this idea received well responses from the three sides. After frequent and deep discussions between me and Prof. Nakano, we finally submitted the JSPS core-to-core proposal as termed as "Research initiative for border transgression of microorganisms: *Microcystis* cyanobacteria as a model microorganisms". In this project, we would like to conduct following researches, with focus on the transportation of *M. aeruginosa* through the bird migration from Indonesia to Japan: 1. Biogeography of *M. aeruginosa* genotypes in Indonesia, China and Japan; 2. Adaptation and evolution of *M. aeruginosa* in lakes we study; 3. Eco-physiological mechanisms on growth of a specific *Microcystis* genotype(s) with particular eco-physiological characteristics, such as toxin production.

2.3 Research progress: a brief report on discovery of saxitoxin producing cyanobacterium in Lake Biwa

My researches have been focused on the diversity and toxicity of water bloom forming cyanobacteria. Searching toxic cyanobacteria in waters is one of the main tasks in my research. Among about 2000 currently existing species of cyanobacteria, many strains from several genera/species are able to produce toxic metabolites called cyanotoxins. Mechanisms of cyanotoxins currently described and understood are very diverse and range from hepatotoxic, neurotoxic and dermatotoxic effects to general inhibition of protein synthesis. Cyanotoxins producing cyanobacteria have mainly reported in planktonic species since they are mostly causative species for water bloom formation in many nutrient-rich water bodies around the world. Compared to planktonic species, there are significant knowledge gaps regarding the distribution, toxin production and species composition of toxic benthic (non-planktonic) cyanobacterial communities, and few studies on toxic benthic freshwater cyanobacteria have been performed despite being linked to numerous animal deaths worldwide. Benthic cyanobacterial mats are found in a range of habitats including wetlands, lake littoral zones, streams and rivers. The development of cyanobacterial mats in fresh waters can occur on a variety of substrates, from bedrock to sand, to artificial structures, and associated with macrophytes. In these mats, the dominant species are usually filamentous Oscillatoriales such as *Oscillatoria, Phormidium, Lyngbya, Leptolyngbya, Microcoleus, Tychonema* and *Schizothrix. Lyngbya wollei* (Farlow ex Gomont) Speziale and Dyck is a large filamentous cyanobacterium inhabiting the benthos of freshwater riverine habitats. It is a known producer of the potent neurotoxic saxitoxins and the taste and odor compounds geosmin and 2-methylisoborneol. This species has a wide distribution including the United States, Canada, southern Europe, India, south-east Asia and north-eastern Australia. However, it has been rarely reported in Japan and China, and its toxin production has never been documented in the Asian region. As I mentioned above, I was performing two surveys for collecting algal samples from Kitayamada, at the south port of Lake Biwa. We specifically concerned the samples with long filaments, and conducted polyphasic characterization of these samples, and

main results were briefly described as below.

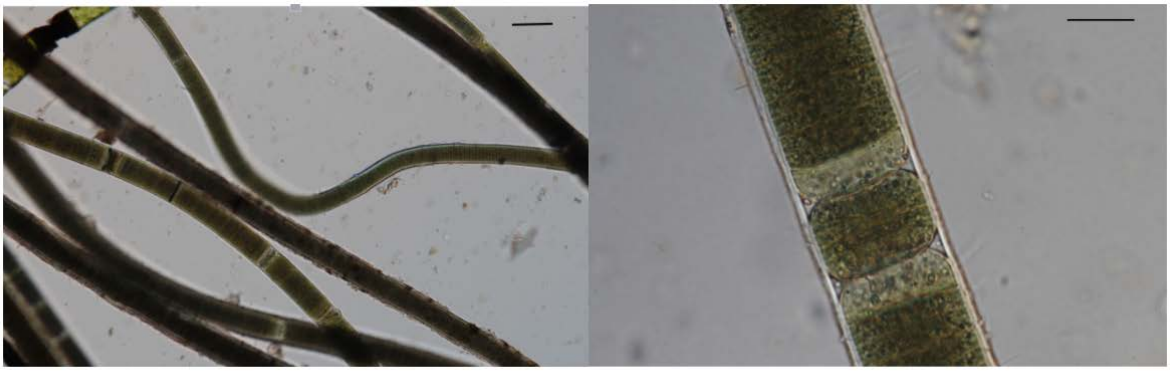

Figure 1. Microscopic images of the sample with filaments of *Lyngbya wollei.* Both bars representing 50 μm.

The samples were dominated by giant filaments with wider cells, with width up to 50μm, and the filaments have very significantly firm sheaths (figure 1). These morphological characters were well

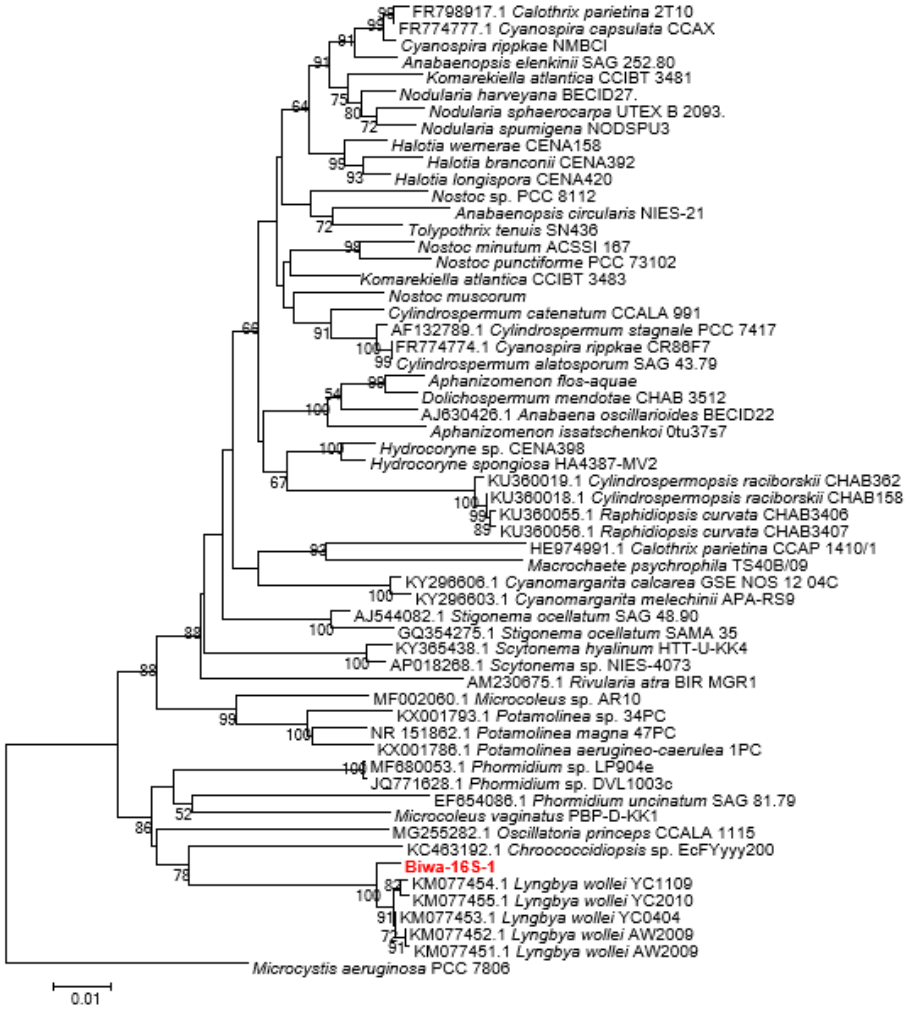

Figure 2. NJ phylogenetic tree based on 16S rRNA gene sequences, bootstrap values with more than 50 shown at each node. *Microcystis aeruginosa* PCC 7806 as the outgroup.

corresponding to those of *Lyngbya wollei* (Farlow ex Gomont) Speziale and Dyck, which were already described above. The genomic DNA was extracted from the sample, and its 16S rRNA gene was amplified and sequenced. The similarity in 16S rRNA based search showed that the closest taxa was *Lyngbya wollei* YC1109，and the phylogenetic tree based on 16S rRNA indicated its gathering with the strains of *Lyngbya wollei*, with a high bootstrap value (figure 2).

To further explore whether the sample has the potential to produce PSP, the PCR on saxitoxin synthesis gene （*sxtA*）was conducted, and the positive amplification was significantly observed. PCR products were sequenced, and the obtained sequences were mostly similar to *sxtA* sequences of *Lyngbya wollei* strains (figure 3).

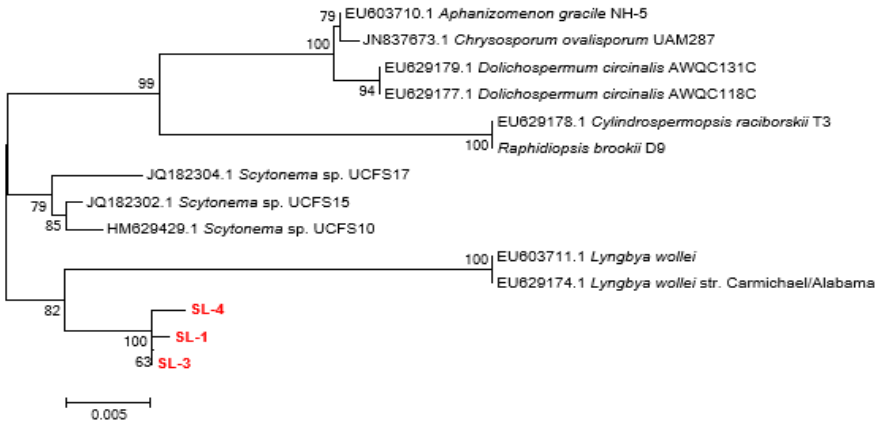

Figure. 3. NJ phylogenetic tree based on saxitoxin synthesis gene (*sxtA*) sequences.

Therefore, the present survey indicated that new toxic cyanobacterial taxa was obviously present in Biwa lake, implying the new trend for the development of cyanobacterial community and their toxicity feature in the lake. Future researches will target on the chemical confirmation of the saxitoxin composition and concentration, isolation of *Lyngbya wollei* strains, and more thorough investigation on *Lyngbya wollei* along the whole Biwa basin. This report is the first discovery of neurotoxic saxitoxin producing cyanobacterium in Asian region.

3. Conclusion

This visit to CER allowed me to have a wonderful opportunity to meet and discuss with many excellent scientists and students at CER and KU, and even in Japan. The whole activities including my research, proposal preparation submitted to JSPS and visit tours to different universities/institutes during my stay were very successful. The brief research report on discovery of saxitoxin producing

cyanobacterium *Lyngbya wollei* in Lake Biwa provided the new trend of harmful algal species and the need to build the new policy for the research, monitoring and management in the lake. The initial suggestion will be to perform the further intensive and extensive studies on this toxic cyanobacterium.

Once again, I would like to express my thanks to the International Research Unit of Advanced Future Studies for the support, and to Prof. Nakano and his colleagues at CER for the helps of my visit.