

## Search for microbial CH<sub>4</sub> production processes in lake sediments and surface water associated with cyanobacterial bloom

\*Makoto Matsushita<sup>1</sup>, Yoshinori Takano<sup>1</sup>, Hiroyuki Imachi<sup>1</sup>, Atsushi Urai<sup>2</sup>, Ho-Dong Park<sup>3</sup>, Hiroki Iwata<sup>3</sup>, Naohiko Ohkouchi<sup>1</sup>

1. Japan Agency for Marine-Earth Science and Technology, 2. Department of Science and Technology, Graduate School of Medicine, Science and Technology, Shinshu University, 3. Department of Environmental Sciences, Faculty of Science, Shinshu University

Methane (CH<sub>4</sub>) is an important greenhouse gas and contributes substantial budget to global warming. Freshwater lakes are identified as one of the main CH<sub>4</sub> sources, as it is estimated that they contribute to 6-16% of natural CH<sub>4</sub> emissions [1]. The main part of CH<sub>4</sub> released from lakes is considered to be produced by methanogenic archaea in anoxic sediments as terminal step in the degradation of organic matter. The microbial CH<sub>4</sub> production in lake sediments has been mainly investigated by microbiological studies using culture-dependent methods and molecular analysis.

Microbial CH<sub>4</sub> production is still regarded by many as a process limited to anoxic environments. Nevertheless, over the past few decades, increasing evidence of CH<sub>4</sub> accumulation in oxygen-saturated lake and marine surface water has emerged [2]. To explain the enigmatic issues, it has been proposed that CH<sub>4</sub> production by methanogenic archaea takes place in anoxic microenvironments such as detritus and animals' gut. Alternatively, recent studies suggest that some bacteria break down methylphosphonate (MPn) or dimethylsulfoniopropionate (DMSP) and release CH<sub>4</sub> as a biogenic byproduct. These novel microbial CH<sub>4</sub> production processes may play a potential contribution to the total atmospheric carbon cycle.

Oxic CH<sub>4</sub> peaks have been found to be closely associated with phytoplankton dynamics across multiple lakes. Grossart *et al.* [3] found potentially methanogenic archaea associated with cyanobacteria such as *Aphanizomenon* and *Microcystis* in oxygenated surface water. Additionally, Berg *et al.* [4] showed that hydrogen produced during nitrogen fixation by cyanobacteria can be consumed by methanogenic archaea. These studies suggest the presence of a CH<sub>4</sub> production process by methanogenic archaea associated with cyanobacteria in lake surface water. However, it is unclear whether the CH<sub>4</sub> production is taking place in actual environments.

In this study, we investigated microbial CH<sub>4</sub> production in sediments and surface water of Lake Suwa in Nagano Prefecture, Japan, a typical hypertrophic shallow lake, using culture-independent methods. Especially, we focused on cyanobacterial bloom and tried to identify a CH<sub>4</sub> production process by methanogenic archaea associated with cyanobacteria. 16S rRNA gene analyses revealed the presence of methanogenic archaea closely related to the order *Methanobacteriales*, *Methanomicrobiales*, and *Methanosarcinales* in sediment core samples from 30 cm depth. On the other hand, 16S rRNA genes and *mcrA* genes of methanogenic archaea were not detected in bulk DNA from cyanobacterial bloom sample (mainly *Microcystis*) [5]. In order to estimate the potential for methanogenic archaea, we extracted Coenzyme F430, a biomarker for methanogenic archaea, from sediment and bloom samples and determined by using HPLC [6]. F430 was detected from sediment samples, but that from bloom sample was currently below detection limit (<1 pmol).

These results suggest that methanogenic archaea actively produce CH<sub>4</sub> in the sediments of Lake Suwa,

however, its contribution in oxygenated surface water seems to be small. We are currently conducting a more sensitive analysis of F430 using LC-MS/MS [6] with the pre-treatment procedure [7]. The analysis may identify the CH<sub>4</sub> production process by methanogenic archaea associated with cyanobacteria in the surface water. Although this study did not cover other CH<sub>4</sub> production processes such as MPn and DMSP decomposition, our results may also be applicable to estimate the *in situ* contribution of these novel CH<sub>4</sub> source.

#### Refs.

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