

Search for microbial CH₄ production processes in lake sediments and surface water associated with cyanobacterial bloom

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Methane (CH₄) is an important greenhouse gas and contributes substantial budget to global warming. Freshwater lakes are identified as one of the main CH₄ sources, as it is estimated that they contribute to 6-16% of natural CH₄ emissions [1]. The main part of CH₄ 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 CH₄ production in lake sediments has been mainly investigated by microbiological studies using culture-dependent methods and molecular analysis.

Microbial CH₄ production is still regarded by many as a process limited to anoxic environments. Nevertheless, over the past few decades, increasing evidence of CH₄ accumulation in oxygen-saturated lake and marine surface water has emerged [2]. To explain the enigmatic issues, it has been proposed that CH₄ 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 CH₄ as a biogenic byproduct. These novel microbial CH₄ production processes may play a potential contribution to the total atmospheric carbon cycle.

Oxic CH₄ 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 CH₄ production process by methanogenic archaea associated with cyanobacteria in lake surface water. However, it is unclear whether the CH₄ production is taking place in actual environments.

In this study, we investigated microbial CH₄ 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 CH₄ 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_4 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_4 production process by methanogenic archaea associated with cyanobacteria in the surface water. Although this study did not cover other CH_4 production processes such as MPn and DMSP decomposition, our results may also be applicable to estimate the *in situ* contribution of these novel CH_4 source.

Refs.

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キーワード：微生物メタン生成、メタンパラドックス、淡水湖、メタン生成アーキア、シアノバクテリア
Keywords: Microbial methane production, Methane paradox, Freshwater lake, Methanogenic archaea, Cyanobacterial bloom