

## Community structure of planktonic bacteria relating to subsurface methane maximum of aerobic lake waters

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Freshwater methanogenesis has become paradox as with the presence of supersaturated methane (CH<sub>4</sub>) in aerobic lake waters. Methane production in oxic lake waters is considered to be produced by planktonic microbes under phosphorus-starved conditions. These microbes having C-P lyase gene are able to produce CH<sub>4</sub> aerobically by utilizing dissolved organic phosphorus (DOP) such as phosphonates under the condition where inorganic phosphorus (P<sub>i</sub>) is extremely limited. However, the metabolic pathways and specific organisms responsible for aerobic methane production are still unknown. Therefore, the study of the community structure and physiology of CH<sub>4</sub>-producing planktonic microbes of freshwater ecosystems are important to fully understand the biogeochemistry of CH<sub>4</sub> in lake ecosystems.

In the present study, we collected water samples at the different depth of nine lakes throughout Japan to examine the existence of subsurface CH<sub>4</sub> maximum in summer. Further, we analysed the community structure of planktonic bacteria in lake water samples through PCR amplification of bacterial 16S rRNA genes followed by sequencing analysis, where aerobic methane production was observed at subsurface maximum of the lakes.

The subsurface CH<sub>4</sub> maximum was observed at thermocline within the upper 10-15m of the nine lakes. The metalimnetic peak of CH<sub>4</sub> ranged between 60 and 600nM among the lakes, corresponding with the dissolved oxygen concentrations and chlorophyll *a* maxima. The amplicon sequencing analyses of bacterial community detected about 7,229 OTUs of bacteria including 58 phyla from epilimnetic, metalimnetic (i.e., thermocline), and hypolimnetic samples of all the study lakes. In thermocline, the relative abundance of Proteobacteria (30%) was dominant, followed by Actinobacteria (20%), Verrucomicrobia (18%), Cyanobacteria (14%), Bacteroidetes (13%) and the others (5%). Among them, Proteobacteria, Actinobacteria, and Cyanobacteria have C-P lyase gene (i.e., *phnJ* gene) in their cells, suggesting that they can utilize phosphonates under P<sub>i</sub>-starved conditions. Moreover, these C-P lyase-carrying planktonic microbes were relatively evenly distributed across the layers of lakes excluding Cyanobacteria, which tended to increase in thermocline where the highest CH<sub>4</sub> peak was observed. The facts suggest that the P<sub>i</sub>-starved planktonic cyanobacteria might have a connection to DOP decomposition including phosphonates, thereby producing CH<sub>4</sub> in oxygenated lake waters. Further research and data processing is still ongoing to identify the specific microbes that could govern aerobic methane production in freshwater lakes.

Keywords: Aerobic methane production, C-P lyase gene, phosphonates, planktonic bacteria

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