A nobel methane production pathway in freshwater ecosystems

*SANTONA KHATUN[1], KOJIMA HISAYA[2], IWATA TOMOYA[1]

1. Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 2. Institute of low temperature science, Hokkaido University

Climate change caused by the increasing of greenhouse gases (GHGs) in the atmosphere is the major problem of the 21st century. Methane (CH_4) is one of the powerful GHGs. Freshwater ecosystems (*i.e.*, lakes and ponds) are recently identified as one of the most important natural sources of atmospheric CH_4, accounting for 18% of total annual CH_4 emission to the atmosphere. It has long been believed that CH_4 is mainly produced by CH_4-producing archaea (i.e., methanogens) in anaerobic lake sediments. However, our laboratory recently revealed the novel CH_4 production by photosynthetic microorganisms (i.e., cyanobacteria) in aerobic lake waters. Similar findings also confirmed that the planktonic microbes in the North Pacific subtropical Gyre have C-P lyase that cleaves the C-P bond of methylphosphonic acid (MPn) and produce methane aerobically as a byproduct of MPn decomposition. However, the pathways and organisms responsible for the aerobic methane production in freshwater ecosystems are still unknown. The ability of CH_4 production by freshwater organisms was examined for ten axenic planktonic microbes by three batch-culture experiments (Experiment 1, 2 & 3). In Experiment 1, to confirm the ability of aerobic methane production ability by planktonic microbes, we compared the CH_4 production of P-starved microbes between inorganic phosphorus (P_i) and MPn addition treatments. In Experiment 2, to identify the enzymatic reaction of aerobic methane production, we measured the CH_4 production of P-starved microbes for various phosphonate addtition treatments (MPn, EPn, 2-AEPn and DMMPn). Finally, in Experiment 3, inorganic nitrogen (N) and P_i was added with MPn to identify the effects of nutrient stoichiometry on aerobic methane production by planktonic microbes. In Experiment 1, the aerobic methane production was observed only in the MPn-add treatment for all microbes, while there was no CH_4 production in control and Pi-add treatments. Therefore, it is confirmed that the most planktonic microbes are able to decompose MPn to produce CH_4 under Pi-starved condition. Experiment 2 also revealed that the test organisms are able to cleave the C-P bond of MPn as a substitute for P_i, thereby producing CH_4 or C_2H_6 gases. However, the Protein BLAST search revealed that the test organisms have no C-P lyase (*phn*) genes, implying that different enzymes may function for the degradation of phosphonates. Therefore, further biochemical and proteomic analyses are necessary to identify the metabolic pathway. Finally, the effect of nutrient stoichiometry on CH_4 production was confirmed in Experiment 3. In particular, CH_4 production rate was accelerated in the MPn+N-addition treatment, indicating that N availability controls the MPn decomposition.
The present study revealed that the planktonic microbes have the ability to produce CH_4 aerobically by cleaving the C-P bond of phosphonates, whereas N availability increases CH_4 production. Therefore, the hitherto unknown CH_4 production by planktonic microbes in aerobic freshwater ecosystems represents a contemporary fact to amend the global CH_4 budget.

Keywords: Aerobic methane production, Planktonic microbes, Freshwater ecosystems, Methylphosphonic acid