

Ecology of kinetoplastid flagellates in freshwater deep lakes of Japan

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Abstract

Introduction

Nanoflagellates are ubiquitous and abundant member of the aquatic ecosystems, playing important role in the matter cycling, and acting as a connecting link between the lower and the higher trophic levels. Nanoflagellates consist of numerous groups of eukaryotic microorganisms having immense functional variability. However, importance of individual group is not known as studies were mainly conducted in natural ecosystems considering them as a whole. Moreover, studies are mainly focused in the marine environments and knowledge about the nanoflagellate ecology is not known from the freshwater ecosystems, especially the deep lakes. Therefore, the aim of the present study is to elucidate the seasonal changes in vertical abundance and composition of nanoflagellate community in a deep freshwater lake, with focus on the ecology of a particular group, Kinetoplastida, and to discuss about their ecological roles.

Materials and methods

Vertical water samples were monthly collected for two years in Lake Biwa, Japan, and also from four other deep lakes of Japan during stratified period, for comparison. Firstly, seasonal nanoflagellate community analysis was conducted from both epilimnion and hypolimnion of Lake Biwa by clone library using universal eukaryote primers. Heterotrophic and autotrophic nanoflagellate (HNF and ANF) abundance was also enumerated using primulin staining. Secondly, abundance of kinetoplastid flagellates in Lake Biwa was enumerated using Catalyzed Reporter Deposition Fluorescent *In Situ* Hybridization (CARD-FISH) with kinetoplastid-specific oligonucleotide probes. Thirdly, the abundance of kinetoplastids from the Japanese deep lakes was analyzed by CARD-FISH. To understand the diversity of kinetoplastids, 454 pyrosequencing was used with both universal eukaryote and kinetoplastid-specific primers.

Results

Abundance of HNF and ANF showed contrasting dynamics in the epilimnion of Lake Biwa. HNF always had higher abundance than the ANF in the water column. However, in the epilimnion, ANF had higher diversity than the HNF in all the seasons. Seasonal variation was observed among the distinct flagellate communities in both the epilimnion and hypolimnion of Lake Biwa.

Kinetoplastids were not detected in the flagellate community analysis using universal eukaryote primers. By contrast, CARD-FISH showed that kinetoplastids dominated in the hypolimnion of Lake Biwa, contributing up to 43.7% of total flagellates.

Dominance of kinetoplastids was also observed in the hypolimnion of all the investigated lakes. For 454 pyrosequencing analysis in Lake Biwa, kinetoplastids

were detected with specific primers, and the dominant OTU had the closest match with *Bodo saltans*. The same OTU was found to dominate in four among the five studied lakes. Moreover, some OTUs with significant contribution were confined to each lake.

Discussion

Contrasting dynamics between the HNF and ANF in the epilimnion of Lake Biwa suggests their differential response to changes in environmental condition. The present study demonstrated seasonal variation of nanoplankton diversity from both epilimnion and hypolimnion, suggesting that previous studies only from the surface waters have missed a large part of the diversity hidden in the deep waters of freshwater lakes. The dominance of kinetoplastid flagellates in the present study showed the importance of the flagellates in matter cycling in the hypolimnion of deep lakes. Community compositions of kinetoplastids in the hypolimnion were similar among lakes with similar trophic status and temperature. This suggests that, community composition of hypolimnion kinetoplastids was shaped according to the environmental condition of each lake. Seasonal changes in the community composition of kinetoplastids were even found within the hypolimnion of Lake Biwa, showing the importance of regular monitoring. The results also indicate that seasonal changes in composition of individual groups are not known properly, and more studies are needed to understand the ecological roles of individual groups. The present study revealed that kinetoplastids were overlooked in the diversity analysis using universal eukaryote primers by both clone library analysis and next-generation sequencing, suggesting that these flagellates were underestimated in the global microbial eukaryote diversity studies using universal eukaryote primers. The

underestimation of a dominant flagellate group indicates that, universal primers might have also overlooked other flagellates. The present study highlighted the usage of group-specific DNA primers, and CARD-FISH probes to understand the ecological role and importance of individual flagellate group.