Elucidation of Dynamics of Prokaryotic and Eukaryotic Microbial Community during Cyanobacterial Bloom

（藍藻類ブルームにおける原核・真核微生物群集の動態解明）

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Abstract

Toxic cyanobacterial blooms frequently occur in many eutrophic freshwater bodies worldwide. The dominant cyanobacteria that cause blooms are *Microcystis* species. Certain strains of *Microcystis* produce microcystin (MCs) as a secondary toxic metabolite, of which many analogs have been characterized. These microcystin analogs (MCs) are harmful to plants, animals, and humans. Moreover, MCs have important effects on the community structure and population growth of zooplankton, which may be the most critical factor for controlling the spatial dynamics of zooplankton.

Up to now, many studies have been carried out to elucidate the impacts of physicochemical parameters on the cyanobacterial growth and MCs production, the MCs degradation mechanism by the *mlr* genes cluster, and the MCs synthesis by *mcy* genes cluster. The correlations among MCs-degrading bacterial *mlrA* gene, MCs-producing cyanobacterial *mcyB* gene, MCs concentration, physicochemical parameters, and microbial community also have been investigated. Some bacteria carrying the *mlr* genes cluster, particularly *mlrA*, are capable of degrading MCs. Yet, the MCs-degrading bacteria may with or without the *mlr* gene (mlr^+ and mlr^- genotypes, respectively). In addition, the bacterial biodegradation of MCs is strongly influenced by bacterial community composition and activity. In addition, the distribution, composition, and interactions of zooplankton might be strongly influenced by cyanobacterial blooms. The predation of cyanobacteria by eukaryotic protozoa and zooplankton (e.g., cladocerans, copepods, and rotifers) represents an important interaction to cyanobacteria during a cyanobacterial bloom. However, most studies of the effects of cyanobacteria on zooplankton have been conducted under controlled laboratory conditions, whereas the natural environment is more complex and less stable, given that cyanobacteria may have different impacts on the short and long-term growth, reproduction, and survival of

zooplankton. Up to the present, limited information is available on the dynamics of the eukaryotic microbial community during the cyanobacterial bloom.

Knowledge of the eukaryotic and prokaryotic microbial community composition, fluctuation the population of the MCs-degrading bacteria and MCs-producing cyanobacteria, MCs concentration, and physicochemical factors in an aquatic environment is important to understand the fate of the bloom and MCs. A thorough understanding will contribute to the development of an efficient warning system to control cyanotoxins at an early stage of bloom. Therefore, the objectives of this study were to link the laboratory study and natural environment and to reveal the correlations among MCs-degrading bacteria, MCs-producing cyanobacteria, MCs concentration, physicochemical parameters, and prokaryotic and eukaryotic community composition in a natural aquatic environment. In addition, the relationships among MCs concentration, cyanobacteria, and potential predators in the eukaryotic community were analyzed.

From the results of this study, the negative correlation between the *mlrA* gene copy number and total MCs concentration (r = -0.468) has been observed, especially from the middle to late stages (during the autumn) of the cyanobacterial bloom, *mlrA* gene copy number and total MCs concentration showed the closely negative relationship (r = -0.664, $p < 0.01$), pointing a dynamic and considerable interaction between *mlrA* gene and MCs concentration. The result suggested a high MCs concentration promoted the increase of *mlrA* gene, indicated that the MCs could stimulate an increase in the abundance of *mlrA* gene, and the degradation of MCs by the mlr^+ might usually occur in the middle or late stages (during the autumn) of the cyanobacterial bloom. As well the one-one relationship between *mlrA* gene copy number and total MCs concentration has been observed, implied mlr^+ genotypic MCs-degrading bacteria was main for biodegradation in this study area.

The results of prokaryotic microbial community based on 16S rRNA gene sequence and eukaryotic microbial community based on 18S rRNA gene sequence revealed a weaker positive correlation between Sphingobacteriales and *mlrA* gene copy number, while Burkholderiales and total MCs concentration showed a weak negative relationship. These findings would be explained by Burkholderiales and Sphingobacteriales might degrade the MCs, both mlr^+ and mlr^- genotypic MCs-degrading bacteria co-existence in the prokaryotic community to biodegrade the MCs. *Microcystis* spp. dominated the prokaryotic community and had a positive relationship with *mcyB* gene copy number (r = 0.351), the relative abundance of Cyanobacteria (r = 0.532, $p < 0.05$), and MCs concentration (r = 0.340). Furthermore, the positive relationship of *mcyB* gene copy number and total MCs concentration (r = -0.678, $p < 0.01$) has been shown. These findings assumed during the cyanobacterial bloom, *Microcystis* spp. was the main MCs producer.

When the highest MCs concentration was reached, predation pressure by Phyllopoda, Copepoda, and Monogononta (rotifers) was reduced; thus, MCs may be toxic to cyanobacterial predators. MCs-degrading bacteria may mitigate the toxicity through MCs degradation because the MCs-degrading bacterial population increased in response to the reduction in the total MCs concentration. In the eukaryotic community, *Bosmina longirostris* which was classified as the small-sized cladocerans was the dominant species (less than the detection limit to 31.5%) as well as showed a close positive relation to pH, Suspended Solids (SS), and dissolved organic carbon (DOC). The strongly positive correlation between *B. longirostris* and Cyanobacteria (r = 0.762, p < 0.01) and *Microcystis* spp. (r = 0.302) was observed in this study area. In addition, the negative trend between total MCs concentration and the relative abundance of *B. longirostris* rather than other species implicated that *B. longirostris* was the main predator to graze the *Microcystis* spp. in this study area. The results of this study suggest that the reduction in *Microcystis* abundance and MCs concentration may be caused by the cooperation between MCs-degrading bacteria and predators.

It is important and meaningful to use biodegradation methods to degrade the MCs. This study links the laboratory study and natural environment, analyzing the correlations among MCs-degrading bacteria, MCs-producing cyanobacteria, MCs concentration, physicochemical parameters, and prokaryotic and eukaryotic community composition in a natural aquatic environment. New insights and essential theory are provided for the early monitoring and reduction of cyanobacterial blooms.

Keywords: Cyanobacterial bloom; *mlrA* gene; *mcyB* gene; Microcystin concentration; Physicochemical parameters; Microbial community