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Community analysis of aggregated bacteria in southern Lake Baikal

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ABSTRACT: The main aim of this study is to unveiling the community structure of aggregated bacteria in lake Baikal and determining the relations with free-living bacteria. For achieving this aim, FISH method was applied to free-living and aggregated bacteria in Lake Baikal at April, 2001. Bacterial counts of free-living bacteria by DAPI staining ranged from 0.2 $\times 10^6$ to 3.2$\times 10^6$ cells·ml^{-1}, which decreased with depths, whereas aggregated bacterial numbers dramatically increased with depths, ranged from 0.4$\times 10^4$ to 3.3$\times 10^4$ cells·ml^{-1}. Also, the ratios of EUB probe binding cells to DAPI counts ranged from 52.3 to 74.1% in free-living bacteria, from 39.6 to 66.7% in aggregated bacteria, respectively. Community composition of aggregated bacteria was very different from free-living bacteria. Especially, that is remarked at 25m depth which is observed the highest value of phytoplankton. The vertical profile of aggregated bacteria community was very particular. β-Proteobacteria was increasing with depth till 100m. In 250m depth, γ-Proteobacteria was 44% of DAPI bound cells, while other groups were less than 1 %. In conclusion, the bacterial community structures of free-living and aggregated bacteria were very different, and they sustain the independent ecosystem separately

Key words: In situ hybridization, aggregated bacteria, Lake Baikal, free-living bacteria;

Introduction

Bacteria can be classified as free-living and attached bacteria in lake ecosystem by habitat condition. The aggregated bacteria are often larger, and are present in higher local concentration and more active on a per-cell bases than free-living bacteria in surrounding bulk water (Griffith *et al.*, 1994), and higher specific exoenzyme activities have also been found with macroaggregates (Karner and Herndl, 1992). Therefore they could have an important role in carbon cycling in aquatic ecosystem.

Recently, new molecular techniques such as fluorescent in situ hybridization (FISH) with group-specific fluorescent-labeled probes are simple and quick to apply for defining bacterial communities (Wagner *et al.*, 1993). The main aim of this study is unveiling the community structure of aggregated bacteria in Lake Baikal and determining the relations with free-living bacteria. For achieving this aim, FISH method was applied to free-living and aggregated bacteria in southern Lake Baikal at April 2001, just after the thawing.

Materials and Methods

Sampling station is situated in the Listvyanichnaya Bay in 2km from Southern Baikal Krestovaya. Samples were collected from 0, 10, 25, 50, 100, 250m depths with a Van-Dorn sampler on April 2001. All samples fixed with 4% paraformaldehyde solution were transported to laboratory with cool condition, and stored at 4°C until further processing.

Analyses of bacterial community were carried out with the FISH(fluorescent in situ hybridization) method by using probes labeled with tetramethylrhodamine to targeted 16S and 23S rRNA. We used the EUB338 probe for domain bacteria, ALF1b probe for α-subclass, BET42A probe for β-subclass, GAM42a probe for γ-subclass of Proteobacteria, CF probe for *Cytophaga-Flavobacterium* group and PLA probe for Plancktomycetales (Amann *et al,* 1992; Manz *et al*, 1992). Detailed methods are described in article of Weiss *et al* (1996).

Results

Bacterial numbers

Counts of free-living bacteria were fairly similar, which were 3.1×10^6 and 3.2×10^6 cells mL^{-1} at 0 and 10m depths, and showed 1.0×10^6 and 1.3×10^6 cells mL^{-1} at 25 and 50m depths, respectively, it showed the minimum numbers 0.2×10^6 cells mL^{-1} at 250m depth. Total bacterial numbers of free-living bacteria decreased gradually with depths. Whereas aggregated bacterial numbers increased with depths, which were ranged from 0.4×10^4 to 3.3×10^4 cells mL^{-1}. The highest abundances were observed as 3.3×10^4 cells mL^{-1} at 250m (Fig. 1).

Bacterial community analysis

Vertical distribution of community structure is shown in Fig. 2. The community structure of free-living bacteria at 0m was very similar to that of 10m. Proportions of β- and γ-Proteobacteria ranged from 12.8 to 13.3% and from 22.5 to 23.1% at 0 and 10m depths respectively. The ratios of β- and γ -Proteobacteria decreased at 25m depth, which were 9.9 and 5.9%, respectively. Both two groups decreased gradually with depths. The α-Proteobacteria, *Cytophaga-Flavobacterium* group and Plancktomycetales were relatively very low proportions, less than 5% between 0 and 50m. But At 100m depth, propor-

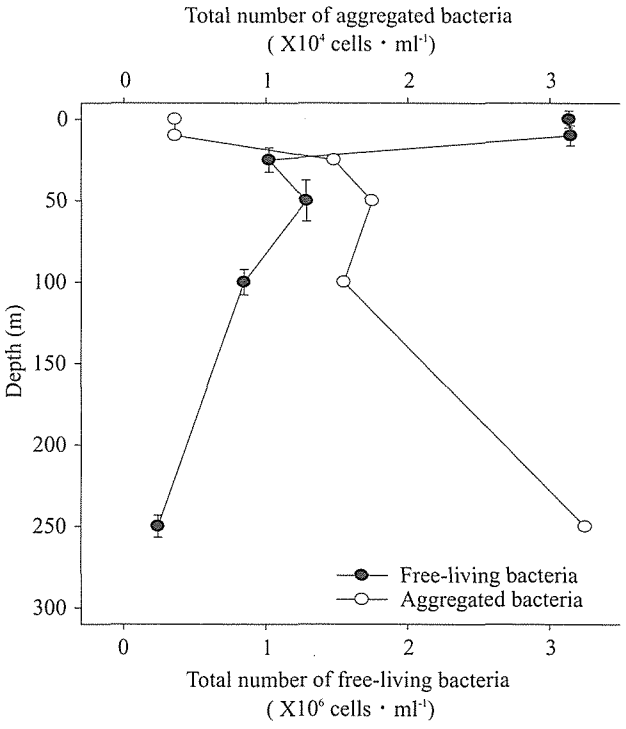

Fig. 1. Vertical profiles of bacterial numbers of free living and aggregated bacteria in Lake Baikal on April 2001.

tions of α-Proteobacteria and Plancktomycetales were slightly increasing by 10.8 and 7.4%, respectively. Community compositions of all groups were similar at 250m depth, which ranged from 1.8 to 6.5%. According to majority of our result, the proportion of Unknown- Eubacteria which is not bound to any probes except EUB338, showed high range from 30.2 to 44.4%, which were slightly increased with depths (Fig. 2). In case of aggregated bacteria, the proportions of all Eubacteria group were ranged from 0.2 to 10.6% at 0 and 10m depths, which were similar to those of free-living bacteria. But at 25m depth, the ratios of β- and γ-Proteobacteria increased in 14.3 and 14.1%, which were different from those of free-living bacteria, whereas *Cytophaga-Flavobacterium* group decreased in 2.2%. Interestingly, β-Proteobacteria drastically increased in 52% at 100m depth but at 250m depth, γ-Proteobacteria dominated as 44%. These structures were fairly different from those of free-living bacteria. The ratios of Unknown-Eubacteria were ranged from 9.1 to 26.3%, which were considerably lower than those obtained from free-living bacteria (Fig. 3).

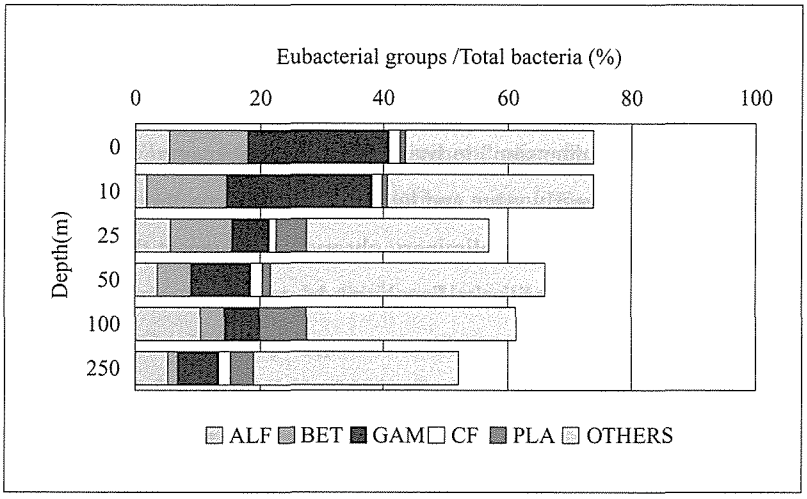

Fig. 2. Community structures of free living bacteria in Lake Baikal at April 2001. (ALF=Proteobacteria α-group, BET=Proteobacteria β-group, GAM= Proteobacteria γ-group, CF=*Cytophaga-Flavobacterium* group, PLA= Planctomycetales, OTHERS=Unknown Eubacteria).

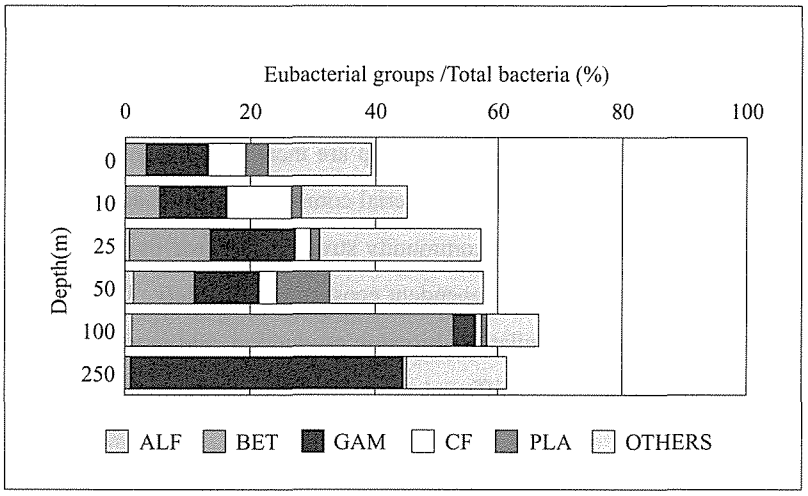

Fig. 3. Community structures of aggregated bacteria in Lake Baikal at April 2001. (ALF=Proteobacteria α-group, BET=Proteobacteria β-group, GAM= Proteobacteria γ-group, CF=*Cytophaga-Flavobacterium* group, PLA=Planctomycetales, OTHERS=Unknown Eubacteria).

Discussion

The relationship between free-living and aggregated bacteria could be hypothesized like following. First hypothesis is that both two bacterial communities are different from each other, and strictly there is no

relationship between two communities. Second hypothesis is that the bacteria occasionally is attaching to newly formed particles and proliferating on the surface. And later, secondary and other invaders cover the microcolony. Most aquatic bacteria attached to nonbiotic and dead particles with "non-specific" mechanism, and "specific adhension" to living material (Bayer *et al*, 1996). Also, on cellulose film, the bacteria reversely attached at early stage and later, bacteria groups are changing with irreversely attaching bacteria (Hong *et al*, 1999). These preliminary observations suggest that the environment of aggregated bacteria may not have close similarities to free-living bacteria with respect to microbial colonization. In this study, the numbers and community structures were quietly different from each other.

The composition of bacterial community could be influenced by attachment substratum, by associated environmental conditions, by other biota such as grazer and organic mater supplier and adhesion ability (Hong *et al*, 1999). With this fact, the community structure could be explained like followings. At 0 to 25m depths, where the chlorophyll *a* value was about 1.5 - 3.1 ug/l, the exudates from phytoplankton stimulate the pelagic bacteria. And to living phytoplankton, the coupling bacteria (Sell, 1993) are attached by 'specific adhesion' mechanism (Fletcher, 1996). And to non- living and dead particles the bacteria attached reversibly. These bacterial communities would start to change into irreversible aggregated bacteria (Vandevivere and Kirchman, 1993). Below 50 m depth, where no phytoplankton exists, and organic materials must come from upper layers and aggregates, numbers of free-living bacteria are decreasing and proportions of Unknwon Eubacteria group are increasing because of limitation of available organic mater and nutrients. But the aggregated bacterial communities are changing by secondary and other invaders.

In conclusion, the bacterial community structures of free-living and aggregated bacteria were very different, and they sustain the independent ecosystem separately. Differences in rates of polymer hydrolysis, substrate uptake and assimilation, biomass production, and predation for attached and free-living bacteria may result in considerably different roles for these communities in material and energy transfer in Lake Baikal.

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