

The Bacterial Community of Southern Lake Baikal in Winter

Tae-Seok Ahn*, Sun-Hee Hong, Dong-Joo Kim,
Jung-Hyun Suck, and Valentin V. Drucker¹

Department of Environmental Science, Kangwon National University, Chunchon 200-701, Korea, and
¹Limnological Institute Russian Academy of Science Siberian Branch, 664033,
Irkutsk, P. O. Box 4199, Russia

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The bacterial abundance, proportion of respiring bacteria, and bacterial community of southern Lake Baikal were analyzed at 1 m and 400 m depths during winter. The total bacterial numbers were 5.1×10^5 cells ml^{-1} at 1 m and 2.5×10^5 cells ml^{-1} at 400 m depth, which are about half and quarter of the numbers of other lakes. The proportion of respiring bacteria was as low as 2.5% at 1 m and 1.4% at 400 m depth. Considering the amount of organic carbon which need to be degraded and low proportion of respiring bacteria, the bacteria could be assumed to have high activities. The EUB/DAPI ratios were 77 and 89% at 1 m and 400 m depths, respectively. Of the bacterial community, the 'other' group was dominant at both depths, and gamma group of proteobacteria followed next. But the beta group of proteobacteria and *Cytophaga-Flavobacterium* groups occupied very small proportions.

Key words: Lake Baikal, bacterial abundance, respiring bacteria, probe, community

Lake Baikal, the largest freshwater ecosystem, accounts for 22% of the world's freshwater supplies, and is the cleanest water body with more than 40 m of transparency. Among the 3,500 species of flora and fauna in Lake Baikal, about 84% are endemic. With these own specific characteristics, there are many taxonomic, phylogenetic, and genetic researches for flora and fauna of Lake Baikal (11), but the phylogenetic studies of microorganisms are rare and recently started. In calculating the amount of organic carbon in Lake Baikal, it was found that about 95% of organic carbon produced by phytoplankton and phytobenthos and input through tributaries and atmosphere are destroyed in water (5), which means that microorganisms play very important role in mineralization and cycling of organic materials and nutrients. But due to their small size and shortage of proper method for identifying the microorganisms, many studies related to microorganisms are restricted to their abundance and physiological properties. And because less than 1% of bacteria in fresh water is culturable, community analysis with cultured bacteria also does not represent the exact bacterial community, so oligonucleotide probes serve as powerful tools for bacterial community analysis (3).

In this study, to preliminarily define the bacterial characteristics of Lake Baikal the bacterial abundance, proportion of active bacteria, and their community during winter are analyzed.

Materials and Methods

Water samples from 1 m and 400 m depths were collected with sterilized water sampler at the South Baikal, near the Listvyanka on December 9, 1998. Just after the sampling on research vessel, the lake waters were incubated with CTC (5-cyano-2,3-ditolyl tetrazolium chloride; Polyscience Europe Inc., Eppelheim, Germany) solution for active bacteria, and fixed with paraformaldehyde solution for community analysis. Total bacterial numbers were analyzed with DAPI staining and epifluorescence microscope (4). Active bacteria were estimated with formation of CTC-formazan, which can be easily detected as red color under epifluorescence microscope (14). Ten milliliters of water sample was added to 1 ml of yeast extract (0.002% w/v) broth and 1 ml of CTC stock solution (5.0 mM). The mixture was continuously stirred for 1 hr in the dark at in situ temperature. After fixing with formaline, the samples were filtered on polycarbonated filter (pore size 0.2 μm) and air dried. Respirometrically active cells were enumerated with epifluorescence

* To whom correspondence should be addressed.
(Tel) 0361-250-8574; (Fax) 0361-251-3991
(E-mail) ahnts@cc.kangwon.ac.kr

microscope.

Bacterial community analysis was carried out with the FISH (Fluorescence In Situ Hybridization) method by using 16S and 23S rRNA targeted probes labeled with tetramethylrhodamine (TaKaRa, Japan). We used the EUB338 probe for domain Bacteria, ALF 1b probe for alpha-subclass, BET42a probe for beta-subclass, GAM42a probe for gamma-subclass of proteobacteria, and CF probe for *Cytophaga-Flavobacterium* group. Twenty milliliters of water samples were filtered through polycarbonate filters (pore size 0.2 μm), and then the filters were reacted with labeled probes in hybridization solution. After hybridization, counter staining with DAPI was carried out and examined with epifluorescence microscopy (2). To reduce non-specific binding with BET and GAM probes, the non-labeled probes were used as competitors (9).

Results and Discussion

Bacterial abundance

Total bacterial numbers were 5.1×10^5 cells ml^{-1} at 1 m depth and 2.5×10^5 cells ml^{-1} at 400 m depth. These values are lower than those of Lake Soyang in Korea and Alpine lakes. In most of these lakes, bacterial numbers were nearly 10^6 cells ml^{-1} . But the bacterial number Lake Baikal was only about half or quarter of the other lakes. This may have resulted from the low temperature and oligotrophic state of the lake. Besides these environmental conditions, the protozoan grazing might be a cause of low bacterial numbers (12). Among the total bacterial numbers, the proportion of respiring (active) bacteria was 2.5% at 1 m depth and 1.4% at 400 m depth (Table 1). In natural conditions, the proportion of actively respiring bacteria detected with CTC to DAPI staining ranged from 6 to 88%; the highest was observed in the ground water and the lowest in seawater (14). But in other marine studies, the proportion ranged from 0.4 to 1.9% (8). Previous studies in which INT reduction (13) was used, the proportions of active bacteria ranged from 4 to 61% in marine (15) and 5 to 36% in freshwater (17). Compared with other data on respiring bacteria, the proportion of active bacteria in Lake Baikal was low and so was the bacterial number.

Table 1. Total bacterial number and respiring bacteria of Lake Baikal in December, 1998

Depth (m)	DAPI count (cells ml^{-1})	CTC positive (cells ml^{-1})	Active bacteria (%)
1	5.1×10^5	1.3×10^4	2.5
400	2.5×10^5	3.6×10^3	1.4

Bacterial community

The ratio of EUB probe binding cells to DAPI counts were 77% at 1 m depth and 89% at 400 m depth. These ratios are similar to those of the natural lakes. In Lake Soyang, the ratio was 30~100% (1). About 35~67% of total bacterial numbers in few ponds (6), 40% of living bacteria in tap water (10), and 50% in oligotrophic lakes in Alps reacted with EUB338 probes (2). But in one of the aeration basin of activated sludge, 89.3% of DAPI stained cells emitted probe conferred fluorescence (16). Among the 4 categories of Eubacteria, the gamma group of proteobacteria was dominant. Gamma group occupied 27% of DAPI counts at 1 m depth and 12% at 400 m depth. Following was the alpha group of proteobacteria. Beta group of proteobacteria and *Cytophaga-Flavobacterium* group occupied only a small proportion. At 1 m depth, both groups were less than 2% of DAPI counts, and at 400 m depth, beta group was only 2% and *Cytophaga-Flavobacterium* group 4% of DAPI counts. The most abundant group was the 'other' Eubacteria as 29.9~62.6%. The 'other' group refers to the bacteria group detectable by EUB, which could not be categorized within the sum of bacteria detected by four group specific probes.

During winter season, in surface layer of Lake Soyang, in a meso-eutrophic lake, alpha group occupied about 11%, beta group 40%, gamma group 9%, *Cytophaga-Flavobacterium* group 9% and 'other' group 31% of the bacterial population. (7). And in pelagic layer of high mountain lakes in winter, beta proteobacteria showed high share of about 43% EUB, and 'other' Eubacteria group 20% (2). The bacterial community of Lake Baikal differed in composition from the above two lakes.

Conclusion

The bacterial number and the proportion of active

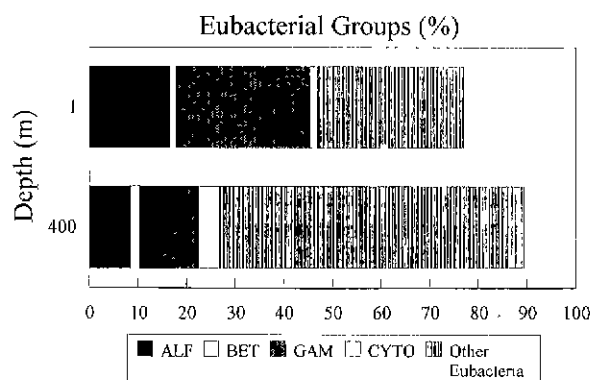


Fig. 1. Bacterial community of southern Lake Baikal in December, 1998. (ALF=Proteobacteria alpha-group, BET=Proteobacteria beta-group, GAM=Proteobacteria gamma-group, CF=*Cytophaga-Flavobacterium* group).

bacteria of southern Lake Baikal were lower than other lakes. Moreover, among the bacterial community, the 'other' Eubacteria group was higher in proportion. This signifies that the bacterial characteristics are different from the other lakes and that it has an unique composition. The water temperature of Lake Baikal is comparatively low. Even during summer surface layer, temperature remains at 17°C in and under ice during late winter. Especially at 400 m depth, the temperature remains at 3.5°C whole through the year (5). Considering the geological age (25×10^6 yr), environmental conditions, and high proportions of endemic species of plant and animal, there may be a lot of specific and endemic species of bacteria, which are psychrophilic, barophilic and oligotrophic. In case of culture collection of *Cyanobacteria* isolated from Lake Baikal and stored in Limnological Institute, Russian Academy of Science Siberian Branch, there are many pink and red ones which are merely found in extreme environment, such as hot spring. There we can conclude that there are many bacteria which possess specific functions, physiological properties, and genes.

As mentioned previously, about 95% of 4,255 Kt of organic carbon produced and input a year need to be degraded inside of Lake Baikal. The proportion of sedimentation was very low, only 3%. And moreover withdrawal by fishing was only 1 Kt, 0.02% (5). And protozoan grazers consumed the autopicoplankton, which contributes a major role in primary production, and 24% of primary production could be attributed to higher trophic level (12). Considering this food web system, high proportion of organic materials which need to be degraded in Lake, and low bacterial numbers and low proportion of respiring cells, we can assume that the respiring bacteria must have high activity for degradation of organic materials. Personally Dr. Parfenova in Limnological Institute SB RAS gave information that most of isolated bacteria from Lake Baikal has high activity for cellulose degradation, alkaline phosphatase, and many others. Unfortunately, we did not measure the bacterial biovolume during our observation, but we could easily distinguish that the size of bacteria are remarkably larger than those from Lake Soyang (data not shown). These results and information suggest that the bacteria in Lake Baikal play important roles in mineralization, food web, and diversity.

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