Distribution and Activity of Sulfate-reducing Bacteria in Lake Soyang Sediments

Ho-Yong Jin, Dong-Hun Lee, Young-Gun Zo, Chan-Su Kang, and Sang-Jong Kim*

Department of Microbiology, College of Natural Science, Research center of Molecular Microbiology, Seoul National University, Seoul 151-742, Korea

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In order to know the extent of contribution to the degradation of organic materials and nutrient recycling by sulfate-reducing bacteria (SRB) and methane-producing bacteria (MPB) in sediment, the distribution and activity of these two groups of microorganisms were studied monthly in 1994 at two sites, one is littoral (Sanggulri) and the other profundal (Dam), in Lake Soyang. In the seasonal distribution of two microorganisms, SRB were 1.07×10^3~2.42×10^5 cells/g-dry weight at Sanggulri, 2.40×10^5~1.29×10^6 at Dam and MPB were 0.52×10^3~5.88×10^3 cells/g-dry weight at Sanggulri and 1.44×10^3~6.89×10^3 at Dam. In these results, the density of SRB in Lake Soyang is much higher than other lakes. These high values might be due to higher sulfate concentration, 0.59~4.05 mM, than normal freshwater, 0.01~0.2 mM. And a good correlation of SRB and chlorophyll a concentration implied that the important environmental factor on distribution of SRB might be the concentration of available organic matter. In a comparison of sulfate-reducing rate and methane producing rate in 1995, the activity of SRB for the degradation of organic matter was higher than MPB by factor of 359. Conclusively SRB superior to MPB in the distribution and activity are more important anaerobic bacteria in Lake Soyang sediments.

Key words: Lake Soyang, sediment, sulfate-reducing bacteria (SRB), methane-producing bacteria (MPB)

Sediment in lake is microbiologically active site where organic matters and nutrients produced from water column are loaded finally. And the clear comparison of viable bacterial number between water column and sediment has led the microbiologists to attend metabolic and ecological potential of sediment bacteria (22). Various environmental factors such as temperature, pH, DO, water depth and organic matter affect the kinds and activities of these sediment bacteria (14) . The sediment bacteria in aquatic ecosystem have three roles following as; one is the oxidation of deposited organic matter, another is the regeneration of inorganic nutrients, and the third is transformations of those inorganic materials (9).

The sediment bacteria are divided into oxygen (O_2)-, nitrate (NO_3)-, manganeous oxide (MnO_2)-, ferrous oxide (FeO_2H)-, sulfate (SO_4^{2-})- and carbon dioxide (CO_2)-respirator by thermodynamically favorable electron acceptors used in the course of degradation of organic matter (28). But oxygen/nitrate, manganeous/ferrous oxide are not suitable for sediment bacteria because of low concentration and solubility, respectively (5, 16, 27). The major bacteria in sediment is SO_4^{2-} and CO_2 respirator named as sulfate-reducing bacteria (SRB) and methane-producing bacteria (MPB) (5).

SRB and MPB have specially important roles in the final metabolic pathway of degradation of organic matter. And these bacteria are generally known for mutual exclusion in space ascribing to the difference of growth yield on acetate and hydrogen substrate and the sulfate concentration (21). But in lake environment, though sulfate concentration is very low, various other factors can affect two bacteria in comparison to marine environment. As a result of these various factors, the distribution and activity of SRB and MPB is different case by case (20, 29).

Lake Soyang is the greatest reservoir in Korea, 100 m deep in maximal depth and 29 billion tons in total storage. And, this lake has been reported the blooms of *Peridinium* and *Anabaena* every year (17, 19) It was reported that 8% of primary productions, ranged from 132 to 589 g-C $m^{-2}yr^{-1}$ in seasonal variation (17), sinked into sediment in this lake

* To whom Correspondence should be addressed

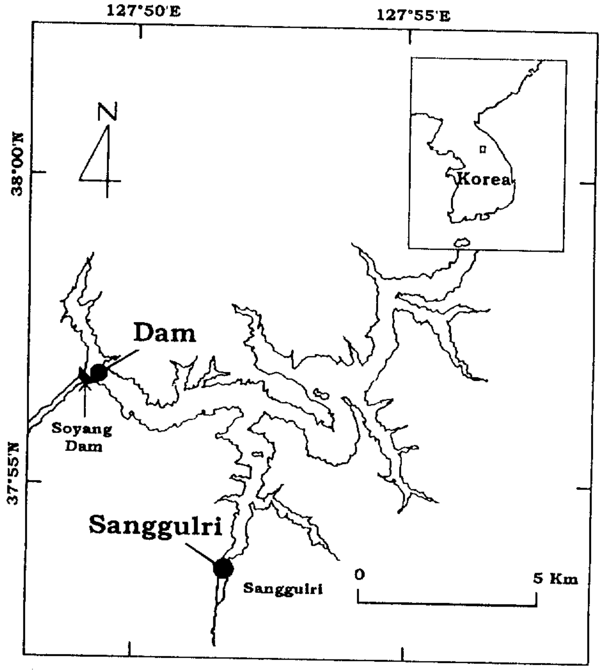

Fig. 1. Map of sampling sites in Lake Soyang.

(6). In addition to this influx, it was reported that the resuspension of sediment annually occurred between late winter and early spring (6). According to these factors, the knowledge of distribution and activity of microbes in sediment is required for more complete understanding of the degradation of organic matter in the lake and nutrients recycling into primary production. And it is expected that anaerobic bacteria play a role in this process in sediment, because high depth and thermal stratification in the summer allow sediment to be in an oxygen-deficient environment. Therefore, this study aimed to investigate the distribution and activity of SRB and MPB which are regarded as the most important anaerobic microorganisms in sediment. And we chose and compared two zones, profundal and littoral, as experimental sites in order to understand the entire lake.

Materials and Methods

Sampling

Sediment samples were collected monthly by a grab sampler from Sanggulri and Dam site, about 1 meter and 80 meter in mean depth respectively, during the period from January to October in 1994 (Fig. 1).

Collected samples were transported to the laboratory in an anaerobic bag (Anaerocult P, merck) to maintain the anaerobic condition.

Analyses of samples

Porosity was calculated by exchanging reduced weight as volume of water, after 1 ml of sediment dried at 70°C for 6 hour (25). Sulfate concentration was analysed by ion chromatography (Hitech, USA) from pore water in sediment, centrifuged at 5000 rpm for 5 min. and filtered with 0.45 μm cellulose mixture membrane filter (Nuclepore Co.). To determine chlorophyll a concentration, after 1 gram sediment was extracted and diluted with 100 ml sterile water by omnimixer (Dupon co.) at 3500 rpm for 5 min, the extractant was diluted again with 900 ml, 200 ml of the diluted was filtered with 0.45 μm cellulose mixture membrane filter, was stored and extracted in 90% cold-acetone for 24 hour and measured the absorbance (2).

The distribution of sulfate-reducing bacteria

First, sediment was mixed well in anaerobic chamber (anaerobic workstation MK-3, Don Whitley). Then, 1 ml of the sediment diluted into 10^{-3} to 10^{-7} and inoculated as triplicate respectively according to dilution scale with sterile water. Postgate B media (24) were used as media for culture with lactate as substrate, and 0.001% resazurin (Fluka) was added as a redox indicator. Inoculated tubes were incubated at 18°C for over 4 weeks and growth of SRB was determined by black precipitate (24). Enumeration of SRB was calculated by 3×3 tube MPN (4).

The distribution of methane-producing bacteria

Sediment was sampled, diluted and inoculated by the same method as sulfate-reducing bacteria. Carbonated-buffered media (30) was used as media for culture with acetate as substrate. After incubation for over 8 weeks, the tube of MPB growth was determined by the concentration of methane over 1 nM. The concentration of methane was quantified by gas chromatography (5890A, Hewlett Packard).

Sulfate-reducing rate and methane-producing rate

After 5 ml sediment and 5 ml distilled water were mixed. This slurry was placed in pressure tube and sealed with a butyl rubber and an aluminum cap. The sealed tube was purged with nitrogen gas through rubber for 5 min. in order to remove oxygen. In dark condition and at room temperature the tube was incubated with shaking of 120 rpm for 38 hours. Sulfate-reducing rate was calculated from the difference of sulfate concentration in pore water before and after incubation. Methane-producing rate was obtained from GC analysis of head space of the tube at 3 hour intervals. Analysis of methane by GC, HP-1 column (column ID : 0.53, Length : 25 m) was used with He as a carrier gas and FID detector under the following conditions: oven temperature 50°C, injector

Table 1. Seasonal variation of porosity, chloropyll a concentration and sulfate concentration in Lake Soyang sediments in 1994

Sampling Date	Prosity		Chlorophyll a (μg/g-dry weight)		SO_4^{2-} (mM)	
	Sanggulri	Dam	Sanggulri	Dan	Sanggulri	Dam
28 Jan.	0.746	-	17.83	-	1.84	-
23 Feb.	0.595	0.741	8.93	49.76	0.84	-
25 Mar.	0.592	0.780	1.35	78.68	1.44	-
22 Apr.	0.543	0.713	3.64	59.11	0.94	0.75
23 May	0.659	0.711	14.47	65.67	0.79	0.78
14 Jun.	0.744	0.841	27.19	139.05	-	0.59
22 Jul.	0.630	0.734	3.72	77.40	4.05	-
26 Aug.	0.422	0.706	0.87	94.56	2.10	0.79
28 Sep.	0.446	0.696	4.18	59.85	1.66	1.45
28 Oct.	0.645	0.817	1.82	38.35	0.84	0.65
Average	0.598	0.747	8.41	73.60	1.61	0.83

temperature 100°C, detector temperature 250°C, flow rate 8 ml/min. Obtained rates were transformed into CO_2 production rate by Aller's equation (1) to compare the activities as unified aspect of the degradation of organic matter.

Results

Seasonal variation of environmental factors

Sediment from Sanggulri site, a littoral zone, appeared to have relatively higher variation of environmental factors than Dam site, a profundal zone, during the studied period (Table 1). Porosity values which showed to be 0.598 (±0.104, SD), 0.747 (±0.049, SD) implicate that a sediment from Dam site was composed of smaller particles than Sanggulri site. High variation at Sanggulri site was resulted from distinctively low values in April, August and September. During these periods the water level reduced drastically and terrestrial soils, which had larger porosity than sediment, entered into sediment at Sanggulri. Perhaps, property of a sediment at Sanggulri site might be changed by these causes. chlorophyll a concentrations at Dam were higher than those at Sanggulri during the studied period. It was in June that chlorophyll a concentration reached the maximum at both sites. And on this month bloom by a diatom was also observed. Sulfate concentrations in a pore water were 1.61 (±0.98, SD), 0.83 (±0.28, SD) at Sanggulri and Dam, respectively. Like other environmental factors, the variation of sulfate concentration also appeared to be higher at Sanggulri than at Dam.

Seasonal distribution of sulfate-reducing bacteria (SRB)

The densities of SRB were 2.4×10^5~1.3×10^6 cells/g-dry weight at Dam and 1.1×10^3~2.4×10^5 cells/g-dry weight at Sanggulri respectively (Fig. 2). Table 2 presents that lakes which have higher sulfate concentration show higher number of distribution of SRB. But, this did not agree with sulfate concentrations which was higher at Sanggulri than at Dam. Variation of sulfate, though electron acceptor of SRB, did not seem to be determinative to the distribution of SRB in Lake Soyang, because it was shown that chlorophyll a concentration (r =0.89, 0.73) rather than sulfate concentration (r<0.35 at two sites) had a better correklation to the distribution of SRB.

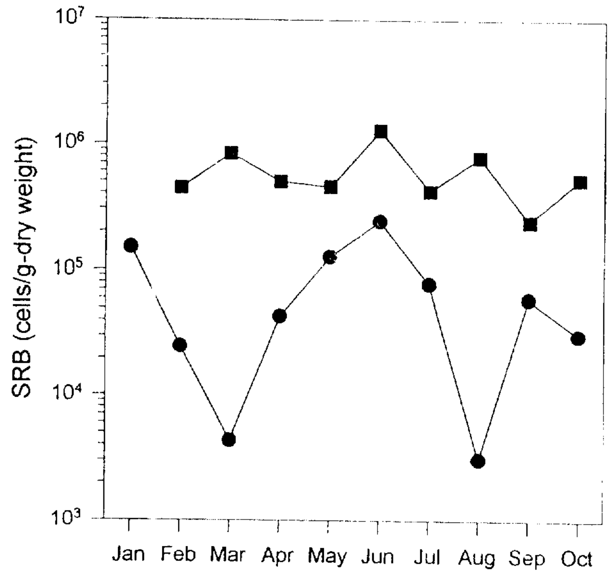

Fig. 2. Seasonal distribution of sulfate-reducing bacteria (SRB) at Sanggulri (●) and Dam (■) in 1994.

Table 2. Comparison of sulfate concentration and sulfate-reducing bacterial number in various lake sediments

Lake	Sulfate concetnrate (mM)	SRB number (cells/ml sediment)	Reference
Lake Teganuma reed site	0.1~3.7	5×10^4-1×10^6	10
Lake Soyang	0.59~4.05	3×10^3-1×10^6	This study
Lake Constance	0.01~0.3	$<5.5\times10^6$	3
Lake Teganuma offshore site	0.05~0.56	1×10^3-5×10^4	10
Bielham tarn	0.05~0.1	5×10^1-2.3×10^3	15
Lake Third Sister	0.028~0.049	3×10^1-7.8×10^2	7
Lake Frains	0.028-0.049	4×10^1-1.6×10^2	7

Seasonal distribution of methane-producing bacteria (MPB)

Seasonal distributions of MPB did not show obvious difference between the two sites but had very low

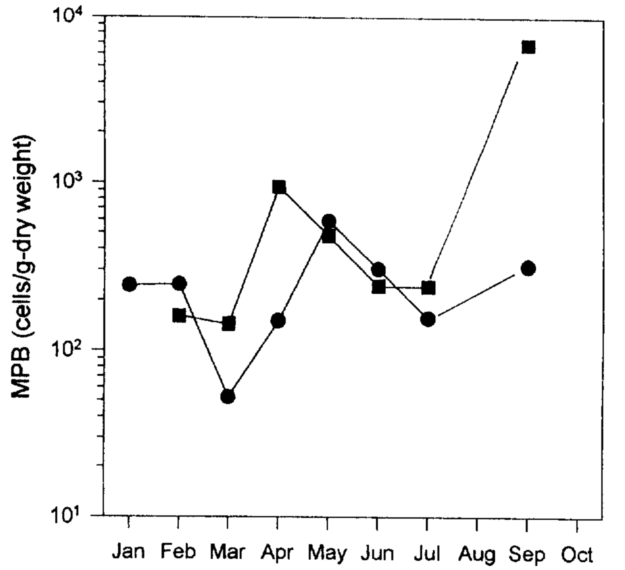

Fig. 3. Seasonal distribution of methane-producing bacteria (MPB) at Sanggulri (●) and Dam (■) in 1994.

Table 3. The comparison of sulfate-reducing rate and methane-producing rate from Dam sediments of Lake Soyang in 1995

Sampling date	Sulfate-reducing rate ($nM-SO_4$/g-dry weight/hour)	Mentane-producing rate ($nM-CH_4$/g-dry weight/hour)
29 Jun.	90.96	1.25
6 Sep.	128.70	0.24
6 Oct.	87.19	0.22
Average	102.28	0.57

values when compared to those of SRB (Fig. 3).

Sulfate-reducing rate and methane-producing rate

Methane-producing rates were 0.003 (June), 0.061 (September) $nM-CH_4$/g-dry weight/hour at Sanggulri sediments and were 0.878 (July)~1.578 (August) $nM-CH_4$/g-dry weight/hour measured from June to October in 1994 (n=5) at Dam sediments. To compare the activities of two anaerobes, MPB and SRB, each activities were measured with Dam sediments in 1995 and calculated as to produced CH_4 or reduced SO4 moles per gram dry weight per hour (Table 3). Obtained values were 0.57 $nM-CH_4$/g-dry weight/hour and 102.28 $nM-SO_4$/g-dry weight/hour respectively. These values were transformed into 0.57 and 204.56 $nM-CO_2$/g-dry weight/hour. As a result, activity of SRB showed 359 times that of MPB and the superiority of SRB to MPB exhibited in the distribution concurred with results of the activity.

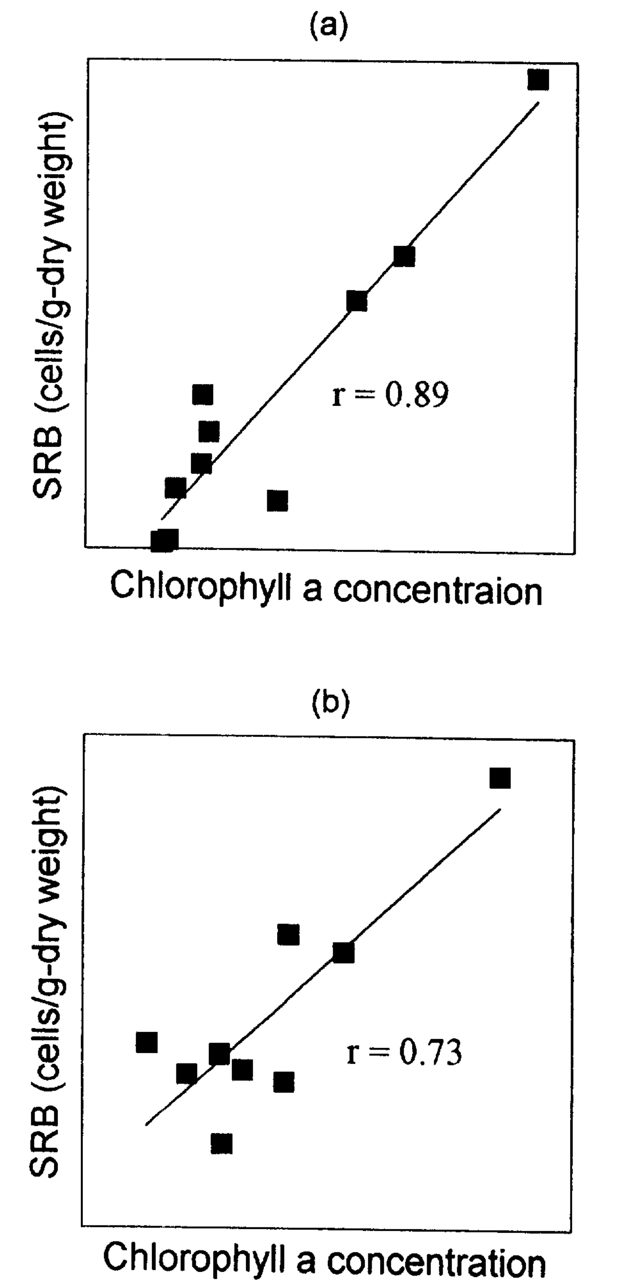

Fig. 4. Correlation of sulfate-reducing bacteria (SRB) and chlorophyll a concentration at Sanggulri (a) and Dam (b) in 1994.

Discussion

The littoral zone of the lake has generally higher variation of environment factors than the profundal zone. Of these factors, physiochemical factors such as temperature, pH and DO show high variation of environmental factors at a littoral zone, owing to shallow depth and closeness to terrestrial soil. In Lake Soyang, high variation at littoral zone, Sanggulri sediments, seems to be due to the change of sediment components by the reduction of water level resulting in the addition of terrestrial soil rather than the influences of temperature, pH and DO. This change was reflected on the variation of porosity. Except January and June, porosity at Sanggulri sediments showed lower values than the range of other lake sediments, 0.7 to 0.8 (18, 25). On the contrary, porosity at Dam sediments had high and stable values between 0.70 and 0.82. Therefore, it is proposed that the changes of physical environment at Sanggulri sediments creates high variation of other environmental factors such as porosity, chlorophyll a and sulfate concentration.

Factors important to the distribution of SRB are temperature, pH, redox potential, available electron acceptors and organic matter (11). Of these factors sulfate as an electron acceptor of SRB, owing to low concentrations ranging from 0.01 to 0.2 mM (31), generally determines the upper limit value of SRB abundance in freshwater sediments. In Lake Soyang sediments, however, sulfate concentrations were higher than the range of general freshwater sediments (Table 1). These high concentrations might cause high abundance of SRB in Lake Soyang.

The source of these abundant sulfate in Lake Soyang sediments is not obvious. But as a possibility we can suppose that the sulfate may be a byproduct of sulfide oxidation of bed rocks. The representative minerals containing sulfur were gypsum ($CaSO_4 \cdot 2H_2O$), epsomite ($MgSO_4 \cdot 7H_2O$) and pyrite (FeS_2) (8). These minerals, if in a heterogeneous metamorphic sedimentary rock layer, could be oxidized with the dissolution of muscovite and carbonate (32). Because most of upper watershed in Lake Soyang consisted of banded biotite gneisis containing a heterogeneous metamorphic sedimentary rock layer (23), surface water and sediment in Lake Soyang could exhibit abundant sulfate.

Another characteristic of SRB distribution is that distributions of SRB were correlated not to sulfate concentration but to chlorophyll a concentration (Fig. 4). The SRB in freshwater sediments have acquired high affinity (low Km) uptake system for sulfate; and sulfate-reduction was independent of the sulfate concentrations down to 0.1 mM SO_4^{2-} (5, 12, 26). And in oligotrophic lakes, sulfate has a tendency to reduce to less for the metabolism of organic matter. Therefore, if sulfate concentrations up to the constant value were preserved in an oligotrophic environment, the distribution of SRB could be dependent not on sulfate concentration but on concentration of available organic matter (20). This rule can apply to Lake Soyang. That is, in Lake Soyang sediments the results showing that distributions of SRB appeared high and were independent of sulfate concentration, implicated that this environment had enough sulfate concentration to sulfate reduction and that the distribution of SRB was limited by the concentrations of available organic matter representing as chlorophyll a.

In addition to the relative abundance of SRB to MPB, low methane production rates during the studied period in 1994 propose that SRB rather than MPB in Lake Soyang sediments play a major role in the degradation of organic matter and nutrient recycling. The comparison of activities of MPB and SRB in 1995 also supports the proposition. Conclusively, SRB in Lake Soyang is more important anaerobes than MPB not only in the distribution but in the activity for the degradation of organic matter.

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