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Image Analysis of Bacterial Cell Size by Diurnal Changes in Lake Soyang, Korea

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To define the effects of zooplankton and phytoplankton to bacteria, bacterial numbers, frequency of dividing cells (FDC) and size distribution were performed with image analysis in the surface layer of Lake Soyang. In August 1992, when *Anabaena* was blooming, the bacterial number increased at daytime. Bacterial numbers and FDC value had a negative correlation ($r=-0.83$, $P<0.01$). Bacterial size spectrums were dynamically changed during the day and night, especially the small bacteria less than 0.5 μm^3. Meanwhile, in October, after the bloom, the bacterial number was only one third of that in August, even though the FDC was higher than that in August. The bacterial numbers of small size class dropped at 13:00. But the size spectrums were relatively constant during the night time. These results suggest that the bacterial growth was tightly coupled with phytoplankton during *Anabaena* bloom. And after the bloom, the bacterial number was controlled grazing activity of zooplankton at daytime.

Key words: Image analysis, bacterial biovolume, diurnal changes, Lake Soyang

Planktonic bacteria constitute an important component of pelagic ecosystem because they are responsible for mineralization of organic matter and cycling of nutrients; and they assimilate low concentration of dissolved organic matter and nutrients into particulate form which then is grazed by predators. And pelagic bacterial production and biomass represent an important link with detritus, low molecular weight of dissolved organic materials and higher trophic levels (6).

Direct microscopic observations are essential for studying the pelagic bacteria. Direct count of aquatic bacteria, such as AODC (10), DAPI staining (21), and Hoechst dye staining (20) are the conventional methods to chase the population dynamics of bacteria, but only in number. Comprehending the bacterial flux of organic matter, and understanding the quantitative importance of bacteria in food webs, a reliable estimation of their biomass was neccessary. But, measuring the biovolume and size using the above methods with a naked eye is labourous, time consuming and sometimes not very accurate. To overcome these disadvantages, an automated and computer assisted image analysis equiped to epifluorescence microscope were applied to aquatic microbial ecology; and this technique was useful to determine the size distribution and the biovolume of bacterioplankton (3, 25, 26).

Bacterial numbers and biovolume would change with water temperature, nutrient availability, physiological state, and growth rate. Moreover, primary productivity and predation could control bacterial growth rate; thus, the dissolved organic materials from phytoplankton and predator are the major effectors to pelagic bacteria. Substantial release of DOC (dissolved organic carbon) from decaying algae, healthy phytoplankton, and excretion and losses from zooplankton grazing stimulates the bacterial growth (4). Grazing pressure of zooplankton (19), cilliate (16), heterotrophic nanoflagellate (15), and protozoa (8) lead to the bacterial loss.

We measured the diurnal changes of bacterial size distribution with Image Analyzer. This study is focused on the bacteria size variations during and after the Cyanobacteria bloom in Lake Soyang, the largest artificial lake in Korea.

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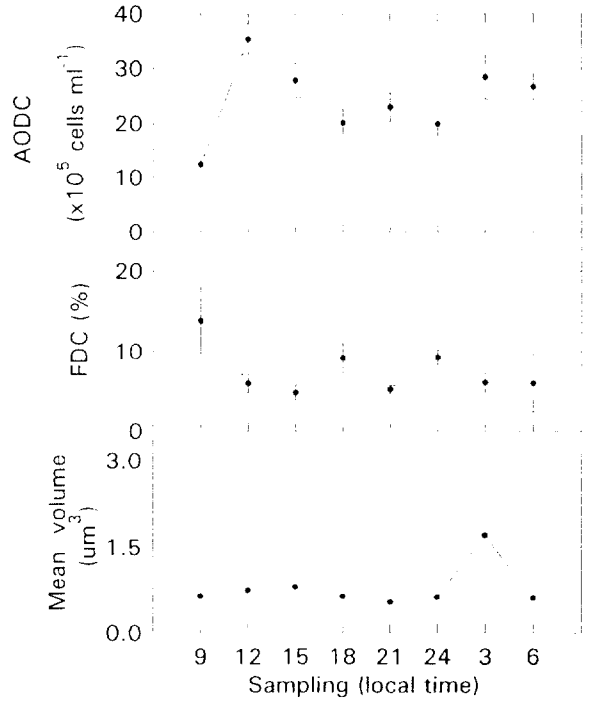

Fig. 1. Diurnal changes in bacterial number, FDC (%) and mean cell volume in the period from 11 to 12, August in Lake Soyang.

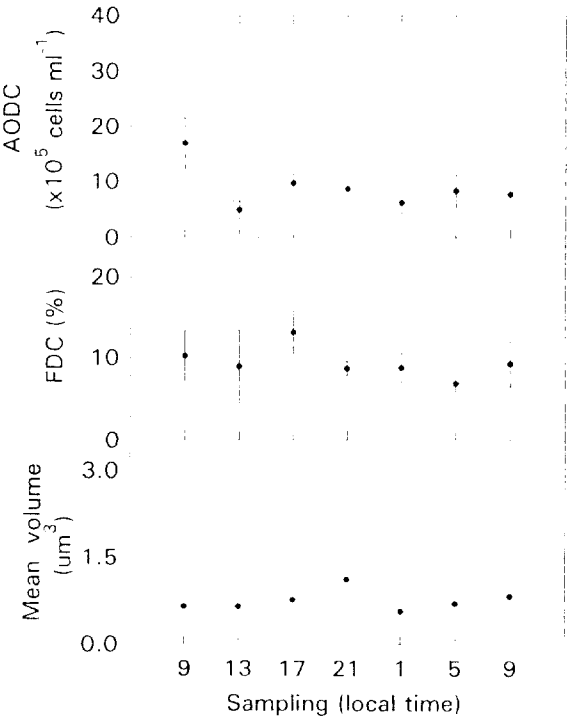

Fig. 2. Diurnal changes in bacterial number, FDC (%) and mean cell volume in the period from 14 to 15, October in Lake Soyang.

Material and Methods

Lake Soyang, the study area, was described in detail by Ahn (1). Since 1988, the Lake Soyang showed several symptoms of eutrophication, such as drastic increase of chlorophyll a content, blooming of *Anabaena* spp. (13) and development of an anoxic zone in hypolimnion (12). Our study area was in the middle of netcage type fish farm. We submerged the vinyl cylinder (ϕ1.5 m×L5 m) to prevent surface water moving. Diurnal variations of bacteria community were measured in August, 11-12, 1992 and in October, 14-15 with 3-4hrs interval.

One hundred mililiter of surface water (0.2 m depth) was fixed with formaline (final concentration 2 % v/v) for Acridin orange direct count (AODC) of Hobbie (10). Frequency of dividing cells (FDC) was estimated with the epifluorescence microscope (9). Sizing of cell parameter was carried out under an epifluorescence microscope (Olympus, BH-2) equipped with an Image Analyzer (Olympus, EX-500) after the technique of Kato (11).

Results

Diurnal changes of bacterial numbers, FDC and mean cell volume

The number of bacteria increased from morning to noon and decreased from noon to evening in August, 1992. In contrast to this, the Bacteria number stayed constant at night and was slightly increased at dawn. Throughout the investigation, the bacterial number ranged from 12.4×10^5 to 35.4×10^5 cells ml^{-1}. Frequency of dividing cells (FDC) fluctuated irregularly. At daytime with the high solar intensity (12:00~15:00), the FDC were low, 5.9 and 4.8 %. Except for the sudden increase at 03:00 to 1.73 μm^3, the mean cell volume was constant, in the range of 0.54~0.81 μm^3 (Fig. 1).

In October, 1992, the number of bacteria dropped from 16.9×10^5 to 5.0×10^5 cells ml^{-1} at 13:00. After this drop, the numbers were constant which ranged from 6.2×10^5 to 9.8×10^5 cells ml^{-1}. FDC ranged from 6.9 % to 13.2 % and FDC at daytime were higher than those in August. Maximum value was recorded at 17:00 as 13.2 % and was constant like the bacterial number. Mean cell volume was similar to that in August. The largest value was observed at 21:00 as 1.11 μm^3. At other times, the mean cell volume was constant, ranged from 0.57 to 0.83 μm^3 (Fig. 2).

Size distribution of bacteria

The size spectrums of bacteria are illustrated in Fig. 3 and 4. In August, the small bacteria less than 0.4 μm^3 were dominant and dynamically changed during the day and night. At noon (12:00), evening (21:00) and dawn (06:00) the small bacteria dominated. The large bacteria, especially the size class of 1.0~2.0 μm^3, were increasing

during the day and at dawn.

Meanwhile, the absolute number of bacteria in October was less than that in August, and during the day the number of small bacteria decreased drastically at 13:00, and then increased again at 17:00. At night, the spectrums were relatively constant.

Discussion

Plankton is the source of the major autochthonous DOC in parallel with DOC provided from sloppy feeding in pelagic ecosystem. The concentration of chlorophyll a is tightly linked to the distribution of bacterioplankton in lakes and seas (2). There is a close relationship between chlorophyll *a* concentrations and bacterial number. The chlorophyll a concentration in Lake Soyang were 7.8±2.8 and 5.2±2.3 mg m^{-3} in August and in October, respectively. By the equation suggested by Bird and Kalff (2), the predicted bacterial number was $36\times10^5\pm16\times10^5$ cells ml^{-1} and $27\times10^5\pm14\times10^5$ cells ml^{-1} in August and in October, respectively. But the estimated bacterial numbers were $23.6\times10^5\pm6.3\times10^5$ and $9.0\times10^5\pm3.6\times10^5$ cells ml^{-1} in August and in October, respectively smaller than the predicted values. Because the water temperatures were 24.9±0.2℃ and 18.1±0.3℃ in August and in October, respectively and since most of the pelagic bacteria are mesophile, the temperature effect might be negligible. So the difference can be explained by grazing. Especially in October, the estimated AODC number was only one third of the predicted AODC number. This shows that the grazing pressure on bacteria was heavier in October than in August.

AODC values and FDC has a negative correlation ($r=-0.83$, $p<0.01$) in August. But there is a 3hr delay on the change of FDC and AODC. Considering the 3hr delay, the FDC and AODC has a strong correlation ($r=0.80$, $p<0.01$). Already high correlation coefficients from thymidine incorporation and FDC had been reported (18, 22). Therefore, these data indicate that bacterial cell division takes place 3hrs interval in August.

Because the bacteria cell size were very widely distributed, from less than 0.1 to 30 μm^3, and the standard deviation was so large, the variations of mean cell volume did not precisely represent the bacterial changes. But, the size spectrum, the number of small bacteria less than 0.5 μm^3 dynamically changed, while the number of bacteria larger than 0.5 μm^3 changed within a narrow range. These results suggest that the image analysis is a powerful tool for understanding the bacterial biomass change.

The bacterial numbers were high during the day in August (Fig. 1). At day time, the number of small bacteria

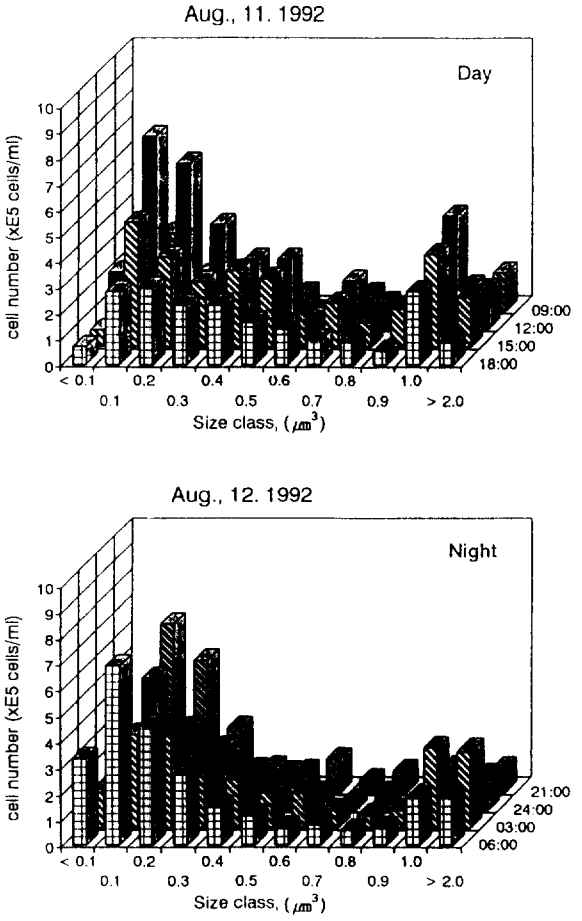

Fig. 3. Diurnal changes of bacterial size spectrum in the period 11 to 12, August in Lake Soyang. Size class 0.2 means that bacteria size are ranged from 0.200 to 0.299 μm^3.

increased at noon, and a proportion of larger bacteria were higher during the day (Fig. 3). Based on this variation, we may conclude that the bacterial growth, both in number and size, is tightly coupled with the excreted organic carbon (EOC) from phytoplankton, especially low molecular weights (LMW). Because of high light intensity the phytoplankton over-produces the CO_2 fixation products which are more than what anabolic turnover requires through the Calvin cycle. So the phytoplankton excret the LMW organic carbon, such as glycolate to eliminate the accumulated organic materials (23). Also in water column the LMW organic carbon, such as dissolved amino acid, dissolved free carborhydrate and glycolate are higher during the light period and lower during the dark period (17). Because of the large amount of this utilizable organic carbon the bacteria become larger and they proliferate more rapidly.

In October, after the cyanobacteria bloom, no relationship is found between the AODC and FDC. Even the FDC percentage at daytime in October was higher

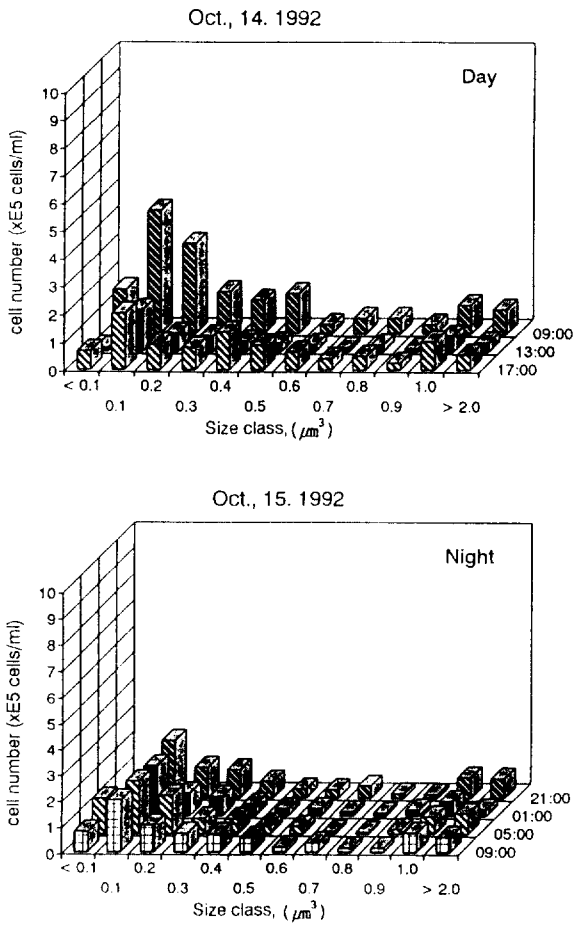

Fig. 4. Diurnal changes of bacterial size spectrum in the period 14 to 15, October in Lake Soyang.

than that in August, the AODC was only one third of that in August. This phenomenon might be caused by the grazing pressure. Because grazing pressure enhances the bacterial activity and selects the fast growth species (r-selected) rather than slow growth species (k-selected) (24), the FDC at daytime was higher (see Fig. 2). So in October, the bacterial growth was not tightly linked to phytoplankton and was controlled by zooplankton. But their size spectrum was not changed during the night while small bacterial number was dynamically changed during the day (see Fig.4). Most zooplankton species migrate upward from deep waters at sunset (28). But in this study area, the zooplankton numbers in daytime is higher than at night (14). Geller *et al.* (7) reported that bacterivorous microzooplankton has high grazing activities during the day compared to the night. Therefore bacteria loss might only occur during the day in October.

The major limnological difference between these two season was the phytoplankton composition. The dominant phytoplankton species were *Anabaena* and *Peridinium* in August, and *Peridinium* in October, 1992 (14). Christoffersen *et al.* (5) reported that ciliate grazing on bacteria constituted 19% in the *Anabaena* bloom period and 39% in the post-bloom period of total grazing on bacteria. Also the macrozooplankton grazed 22% of bacterial production during the bloom and 89% after the bloom. This means that the bacterivorous zooplankton were becoming dominant by the presence of some chemical released during the Anabaena degradation. In August, 1991, in the late stage of *Anabaena* blooming, most of macrozooplankton of this study area were bacterivore (27). And *Bosmina* spp was bacterivore during the late stage of *Anabaena* bloom, and algaevore when diatom was dominant. These results may suggest that *Anabaena* regulate the bacteria number and biomass through the zooplankton grazing.

It is not clear, however, that the diurnal and sesonal changes in cell volume were due to either a change in bacterioplankton species composition or a change of cell volume within the same species. Therefore, in the future, the bacterial diversity should be studied to get more information of bacterial changes in aquatic ecosystem.

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