Vertical Distribution of Free-living and Attached Heterotrophic Bacteria in Lake Kizaki

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Abstract

The form of heterotrophic bacterial occurrence in an aquatic environment was observed by the use of a differential filtration method. Free-living heterotrophic bacteria (obtained in a 1 μ m Nuclepore filtrate) were dominant in the epilimnion of a mesotrophic lake with a maximum depth of 29.5 m irrespective of the high amount of suspended particulate organic matter during the summer stratification period. In the hypolimnion, however, attached bacteria (involved in a fraction larger than 5 μ m) were found to be relatively abundant. A correlation was found between the ratio of free-living heterotrophic bacteria to total heterotrophic bacteria both in number and the dissolved organic carbon (DOC) concentration of lake water. More than half of the heterotrophic bacteria in many of the water samples with DOC higher than 0.7 mgC·l⁻¹ were in the free-living form.

1. Introduction

Heterotrophic bacteria, which play an important role in the decomposition and consumption of organic matter in natural waters, have been recognized to occur either as freely floating cells or attached to suspended particles or debris. Although this difference in the form of bacterial occurrence in natural waters seems to be important in their utilization or decomposition of organic matter, little attention has been paid to the causal relation between their distribution pattern and organic matter utilization. Several researchers have reported that in organic carbon-poor water bodies such as open ocean and oligotrophic lakes, heterotrophic bacteria occurred mostly attached to particles (ZOBELL, 1943; SEKI, 1972; TAGA and MATSUDA, 1972; PAERL, 1975). Particles may provide a solid surface suitable for bacterial colonization and for concentrating substrates on their surface from solution (STOTZKY, 1967). However, FERGUSON and RUBLEE (1976), AZAM and HODSON (1977) and RIEMANN (1978) found that in near shore and coastal waters with eutrophic characters bacteria occurred mostly as free-living cells. These results suggest that the form of bacterial occurrence in natural water bodies may be greatly influenced by the concentration of

organic substrates in water as well as other environmental factors.

GOULDER (1976) and ISHIDA *et al.* (1980) showed that there was a good correlation between the abundance of attached bacteria or a kind of attached bacteria and concentration of particulate matter in an estuary and lakes. Based on such information, the present authors examined the vertical distribution pattern of heterotrophic bacterial occurrence form and its relation to organic matter in a stratified lake as a part of studies on the bacterial function through the organic matter metabolism in a lake ecosystem. Lake Kizaki, a typical dimictic lake in Japan, was selected for the present study. It is a mesotrophic lake with a maximum depth of 29.5 m. Observations in this lake have revealed a seasonal variation in the vertical distribution of both heterotrophic bacteria and organic matter (KATO and SAKAMOTO, 1979). Such variation might be accompanied by a change in the organic matter-bacteria relation.

2. Materials and Methods

Water sampling for bacteria and organic matter was carried out at the center of the lake during the summer stratification period of 1978. Water temperature was measured using a thermistor, and water samples for chemical analysis were collected

with a Van Dorn water sampler (3l). For bacterial works, a sterilized grass ampoule sampler was used (TERAI, 1979).

Soon after sampling, each water sample for chemical analysis was filtered through a pre-ignited (at ca. 450°C for more than one hour) Whatman GF/C glass filter. The filters and filtrates were frozen at -20°C till analysis. Weight of particulate matter (PM) was determined as the difference in dry weight of filters dried at 110°C prior to and after filtration. Chlorophyll-*a* was measured by the method of UNESCO (1966). Particulate organic carbon (POC) retained on glass fiber filter was determined with a CHN analyzer (Yanaco Model MTS-2). The amount of PM, Chl. *a* and POC smaller than 5 μm was determined with the samples prefiltered through a 5 μm nylon net (Nytal HD-5, Swiss Silk Bolting Cloth Co., Ltd.). Dissolved organic carbon (DOC) was measured by the technique of MENZEL and VACCARO (1964) on the filtrates of GF/C filter.

The number of viable heterotrophic bacteria was determined by the pour plate method using one-tenth strength of nutrient agar medium consisting of 1 g polypeptone, 1 g meat extract, 0.5 g NaCl and 18 g bacto-agar in 1000 ml distilled water (pH 7.0-7.2), since this medium always gave a larger number of bacteria than an undiluted medium. Incubation was done at 20°C for epilimnetic samples and 6°C for hypolimnetic samples. After the incubation of 3 weeks, a colony count was made for each triplicated petri dish under a microscope ($\times 10$).

To enumerate numbers of free-living bacteria and attached bacteria, lake water samples were filtered through a 1 μm Nuclepore filter (SHELDON, 1972) and a 5 μm nylon net. The viable bacterial numbers in the filtrates were determined as described above. The heterotrophic bacteria in the 1 μm Nuclepore filtrate were defined as free-living bacteria according to FERGUSON and RUBLEE (1976) and RIEMANN (1978). The difference between the total number of viable heterotrophic

bacteria in unfiltered sample and the viable bacterial number in the filtrate passing through a 5 μm nylon net was tentatively considered to be the attached bacteria, based on the information concerning the average size of phytoplankton and detritus (WILLIAMS, 1970; PEARL, 1975; GOULDER, 1977). The microphotographic analysis using scanning electron microscope indicated that free-living, clumped and attached bacteria were involved in the fraction of 1-5 μm . Thus the sample of this fraction was excluded from the present study to clearly characterize the form of bacterial occurrence of two types (free-living or attached) and to scrutinize their relation to organic matter. In every case, water samples for bacterial works were well blended for 3 minutes with a Thermomixer (Thermonics Co., Ltd., Tokyo) before incubation so as to disperse aggregates and attached bacteria. Three dispersion procedures were tested. Throughout the blending with a Thermomixer, ultrasonication (Bransonic-12, Yamato, Tokyo) and Tween 80 treatment after JANNASCH and JONES (1959), a constant blending procedure with the Thermomixer for 3 minutes gave the maximum and reproducible bacterial number.

Care was taken in the filtration of lake water so as not to destroy detritus and bacterial aggregates. The filtration pressure did not exceed 10 mmHg. For each filtration, a pre-sterilized disposal syringe (10 ml capacity) and filter holder (Millipore Swinex[®]) with a Nuclepore filter or Nytal net were employed as the filtration apparatus.

3. Results

Vertical profiles of water temperature and the number of total heterotrophic bacteria (colony forming units (CFU) $\cdot \text{ml}^{-1}$) in 1978 are presented in Fig. 1. Based on the vertical profile of water temperature, the hypolimnion deeper than 10 m depth can be clearly separated from the upper layer throughout the period of observation.

The number of total heterotrophic bac-

Fig. 1. Vertical profiles of water temperature and total heterotrophic bacteria in 1978.

Fig. 2. Vertical profiles of the ratio of free-living (dotted) and attached (hatched) heterotrophic bacteria to total heterotrophic bacteria in viable number.

teria in the epilimnion in this time varied from 1.2×10^2 to 2.6×10^3 CFU·ml⁻¹. The largest densities appeared at the subsurface or just above the thermocline. The epilimnetic annual maximum number was found in August. The number of bacteria decreased sharply through the thermocline and attained to 0.8×10^1 – 2.5×10^2 CFU·ml⁻¹ in the hypolimnion. The minimum number of bacteria was found at 15 to 20 m depth, although the number increased in the deeper layer, especially above the mud surface. This may have been due to a water-mud interaction or a disturbance of the mud at the sampling time.

Figure 2 shows the vertical change in the ratios of the number of free-living and

attached heterotrophic bacteria to total number of heterotrophic bacteria. The abundance of free-living bacteria was clearly evident in the epilimnion. In the epilimnion, the mean value of the ratio of the number of free-living bacteria to that of total heterotrophic bacteria was 0.56 and that of attached bacteria was 0.21. In the hypolimnion, where abundant attached bacteria were frequently encountered except for November, the ratio of attached bacteria was 0.34 whereas that of free-living bacteria was 0.20.

Figure 3 shows the vertical distribution of PM, Chl. *a*, POC and DOC in 1978. Their amounts for a fraction larger than 5 μ m were large in the epilimnion, espe-

Fig. 3. Vertical profiles of PM, Chl a, POC and DOC.
 (□), Total particles. (■), Particles less than 5μ . (○), DOC.

cially in June. Throughout the metalimnion, their amounts for a fraction larger than $5\text{ }\mu\text{m}$ decreased sharply with depth. For both $5\text{ }\mu\text{m}$ fraction and unfractionated samples, the increment of PM and POC at the deeper layer near the bottom may be due to mechanical turbulence of the sediments.

The DOC level was $0.41\text{--}1.98\text{ mgC}\cdot\text{l}^{-1}$ throughout the observation period, and its vertical profiles were not so different from those of POC.

4. Discussion

The present study indicated that in Lake Kizaki, the ratio of the number of free-living heterotrophic bacteria to total heterotrophic bacteria was generally large in the epilimnion and decreased with depth. On the other hand, more attached bacteria were observed in the hypolimnion than in the epilimnion. As to the form of bacterial occurrence in natural waters, it was suggested that heterotrophic bacteria tend to attach to particles in the ocean environments where the concentration of organic matter is too low for bacterial growth (SEKI, 1972; PAERL, 1975). On the contrary, the dominance of free-living bacteria was observed in organic matter rich waters (RIEMANN, 1978; FERGUSON and RUBLEE, 1976). Unfortunately, none of these previous studies has shown a direct relation between the quantity of organic matter and the form of bacterial occurrence.

In Lake Kizaki, amounts of both particulate and dissolved organic matter were relatively large in the epilimnion where most heterotrophic bacteria occurred in the free-living form. Therefore, particles larger than $5\text{ }\mu\text{m}$ in the epilimnion, which seemed to consist chiefly of the living phytoplankton, were thought to have less attached heterotrophic bacteria. In the hypolimnion, where organic matter particles larger than $5\text{ }\mu\text{m}$ were not abundant, however, heterotrophic bacteria in a form attached to particles or in the aggregated state were rather abundant. Statistically, however, a good correlation was not observed between the abundance of attached heterotrophic bacteria and PM ($>5\text{ }\mu\text{m}$) or POC ($>5\text{ }\mu\text{m}$) in the present study. The difference in sampling time for the bacterial investigation and chemical analysis may be one reason for the inconclusive results.

To clarify the dependence of free-living heterotrophic bacteria on DOC, the ratio of free-living heterotrophic bacteria to the total heterotrophic bacteria in number was plotted against DOC values in Fig. 4. With increase in the amount of DOC, the proportion of free-living bacteria increased. The ratio was correlated with the amount of DOC at a 5% significance level ($n=25$, $r=0.845$). An exception was encountered in the samples from the deeper hypolimnion with high DOC concentration. This might reflect the low activity of microheterotrophs under oxygen depletion and low tempera-

Fig. 4. Relationship between DOC and the ratio of the number of free-living heterotrophic bacteria to that of total heterotrophic bacteria. (○), Epilimnetic samples. (●), Hypolimnetic samples.

ture, or the liberation of DOC from lake mud. It must be also noticed that more than half of the heterotrophic bacteria were in the free-living form in the water with a DOC concentration higher than $0.7 \text{ mgC} \cdot \text{l}^{-1}$.

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摘 要

水中の細菌を存在様式によりグループ分けすることは、水界の有機物（溶存態・懸濁態）代謝に関与する従属栄養細菌の役割を明らかにする上で、有効な方法ではないかと考えられる。その第一段階として、水中の有機物量と従属栄養細菌の存在様式との関係を中栄養湖の木崎湖で調べた。観測結果より、表水層では、植物プランクトン等多量の懸濁粒子が存在するにもかかわらず、浮遊生活細菌が優勢であり、深水層では、懸濁粒子付着細菌の占める割合が大きくなった。溶存有機物濃度と、全従属栄養細菌に占める浮遊生活細菌の割合との間に相関がみられ、浮遊生活細菌が全従属栄養細菌の半数以上を占めるというケースは、DOC $0.7 \text{ mg} \cdot \text{l}^{-1}$ 以上の水塊で観測された。

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