Note

Enrichment of *Macromonas* sp. Densely Populating an Upper Boundary of the H₂S Layer of Lake Kaiike

Michiro MATSUYAMA

Abstract

Two large-celled bacterial species, *Macromonas* sp. and *Chromatium* sp., densely populate an upper boundary of the H₂S layer of Lake Kaiike (bacterial plate). Growth of *Macromonas* sp. was examined from the laboratory incubations of the bacterial plate samples under different conditions. Enrichment of the bacterium was achieved in a medium of DUBININA and GRABOVICH (1984) which contained CH₃COONa, casamino acids and yeast extract. H₂S was inhibitory for the bacterial growth. Without addition of such organic matter into the bacterial plate sample, the bacterium could grow as long as *Chromatium* sp. existed in considerable quantity, and growth of *Chromatium* sp. was H₂S-limited.

Key words: *Macromonas* sp., enrichment in organic medium, Lake Kaiike

Two kinds of slowly moving bacteria populate an upper boundary of the H₂S layer of Lake Kaiike so densely that the boundary is colored purplish red (bacterial plate). They have a striking morphology characterized by intracellular bodies. One is an ovoid phototrophic bacterium (about 5×8 μm) containing numerous sulfur globules (MATSUYAMA, 1987a). Another is bean-shaped (about 3×7 μm), contains 2 or 3 large pearl white refractile inclusions and closely resembles *Macromonas bipunctata* (BAVENDAMM, 1924; SKUJA, 1956). These bodies increase the cellular buoyant density of both species higher than 1.26 g·cm⁻³ in spite of their planktonic life (MATSUYAMA, 1991). The two species could grow as long as they contain the bodies (MATSUYAMA, 1988). In this study, these two species are referred to as *Chromatium* sp. and *Macromonas* sp., respectively.

Macromonas species, comprised of *M. mobilis* and *M. bipunctata* (WATSON, 1974), was grouped into chemolithotrophic colorless sulfur bacteria, merely because the species was generally

encountered in natural habitats characterized by simultaneous presence of O₂ and H₂S (LA RIVIÈRE, 1965). The large pearl white inclusions, which are also found in another colorless sulfur bacteria, *Achromatium* species, are supposed to be CaCO₃, and these inclusions are suggested to exert a buffering effect on H₂SO₄ formed by sulfur oxidation (DE BOER *et al.*, 1971; LA RIVIÈRE and SCHMIDT, 1981).

Reports of occurrence of *Macromonas* species in natural waters are limited (BAVENDAMM, 1924; SKUJA, 1956; LACKEY and LACKEY, 1961; GORLENKO and LOKK, 1979). The ecology of this species has remained quite unknown, because no species had been grown in pure or mixed cultures in the laboratory except DUBININA and GRABOVICH (1984). To the author's knowledge, such a dense bacterial population at the H₂S boundary of Lake Kaiike reaching a level of 1 × 10⁷ cells·ml⁻¹ has not been reported for other natural waters. An attempt by the author to enrich and isolate *Macromonas* sp. by applying various inorganic media failed.

DUBININA and GRABOVICH (1984) purely iso-

lated the three strains of *Macromonas bipunctata* from the precipitates of sewage aeration tanks, and they found that all strains were heterotrophs using a limited number of externally supplied organic carbon (mainly organic acids). The strains were strictly aerobic and aerophilic, and reduced sulfur compounds were

not used as electron donors: their oxidation was due to interaction with H_2O_2 , which was the main product of O_2 reduction in respiration.

The present study aims to examine the growth of *Macromonas* sp. from the laboratory incubations of the bacterial plate samples under different conditions.

Fig. 1. Microphotographs of the bacterial plate collected on 10 April 1990 (A), and the sample collected on 10 August 1990 which was incubated for 18 days in 65-ml glass bottles daily added with different amounts of the medium of DUBININA and GRABOVICH (1984) (B-F). At a minimum rate of addition of the medium ($0.05 \text{ ml} \cdot \text{bottle}^{-1} \cdot \text{d}^{-1}$) growth of *Macromonas* sp. was most marked and the bacterium was dominant (B). But at a rate of $0.1 \text{ ml} \cdot \text{bottle}^{-1} \cdot \text{d}^{-1}$, a small coccoid bacterium became dominant (C), and above the rate sulfur-bearing *Chromatium* sp. (D), *Lamprocystis roseopersicina* (E) and rod-shaped bacterium forming aggregates (F) appeared in considerable numbers.

Fig. 2. Vertical profiles of *Macromonas* sp. (A), *Chromatium* sp. (B) and some environmental factors in Lake Kaiike during 12 December 1987 and 8 August 1990.

Figure 1A is a microphotograph of the bacterial plate sample collected from the H_2S boundary of Lake Kaiike on 10 April 1990. *Macromonas* sp. and *Chromatium* sp. were dominant. Other microorganisms such as planktonic algae were fewer than 10^4 cells·ml⁻¹. However, the relative abundance of the two bacterial species changed with the season.

Figure 2 shows the vertical profiles of the two bacterial species as well as some environmental factors in Lake Kaiike during December 1987 and August 1990. The apparatus and methods used for this study were already described (MATSUYAMA, 1985a). When the temperature at the H_2S boundary was below 30°C, the maximum numbers of the two species were 10^5 – 10^6 cells·ml⁻¹, respectively. When the temperature exceeded 30°C (October 1989 and August 1990) *Macromonas* sp. increased to 4×10^6 – 1×10^7 cells·ml⁻¹, while *Chromatium* sp. were few in number. Laboratory experiments on the growth of *Chromatium* sp. at different temperatures showed that the optimum temperature was 30–32°C (specific growth rate = $1.0 \cdot d^{-1}$), but just above this temperature the bacterium showed a negative growth rate (MATSUYAMA, 1987b). *Chromatium* sp. at the H_2S boundary in October 1989 and August 1990 seemed to be dying due to an extremely elevated temperature.

A bacterial plate sample collected on 8 August 1990 was poured into a 1-l glass bottle and transported to the laboratory while keeping it cool and dark. The sample was centrifuged (15 min, $1000 \times g$), and most of the overlying water was removed. Bacterial cells deposited on the bottom were homogeneously dispersed into filter-sterilized seawater containing the trace element solution of PFENNIG and LIPPERT (1966) ($10 \text{ ml} \cdot l^{-1}$) and vitamin B₁₂ (final concentration = $1 \mu g \cdot l^{-1}$) as indicated by DUBININA and GRABOVICH (1984). Each 40 ml of inoculated seawater was poured into six 65-ml glass bottles and capped with porous silicon rubber stoppers. Eighteen other bottles were filled with inoculated seawater for subsequent anaerobic incubation added with H_2S , and these bottles were tightly closed with screw caps.

All bottles were incubated in a water bath at 25°C for 18 days. All bottles were opened daily and different amounts of neutralized H_2S solution having a concentration of $280 \text{ mgS} \cdot l^{-1}$ were added (0–2.0 ml per bottle) along with the medium of DUBININA and GRABOVICH (1984) (0–1.0 ml per bottle) which had the following ingredients ($g \cdot l^{-1}$): CH_3COONa 8, Difco casamino acids dehydrate 0.8 and Difco yeast extract 0.8.

Figure 3 shows the cell number of *Macromonas* sp. in each bottle at the end of incubation. Cell numbers of two phototrophic bacterial species, *Chromatium* sp. and *Lamprocystis roseopersicina*, as well as H_2S concentration measured by the method of CLINE (1969), are also given in the figure. Without additions of H_2S and the medium of DUBININA and GRABOVICH (1984), *Macromonas* sp. could keep its initial cell number (2×10^5 cells·ml⁻¹) until the end of incubation. Addition of the medium, however, enriched the bacterium: the number attained the maximum level when the medium was added at a minimum rate ($0.05 \text{ ml} \cdot \text{bottle}^{-1} \cdot d^{-1}$) (Fig. 1B). But at a rate of $0.1 \text{ ml} \cdot \text{bottle}^{-1} \cdot d^{-1}$, a small very motile coccoid bacterium became dominant (Fig. 1C) and trace amount of H_2S appeared. Above this rate, sulfur-bearing *Chromatium* sp. (Fig. 1D), *Lamprocystis roseopersicina*, a gas-vacuolated species which sporadically bloomed at the H_2S boundary of Lake Kaiike (MATSUYAMA, 1985b) (Fig. 1E), and a rod-shaped bacterium forming aggregates (Fig. 1F) (a brown *Chlorobium* species?), appeared in considerable numbers. External addition of H_2S inhibited the growth of *Macromonas* sp. The bacterium is suggested to be heterotroph rather than a chemolithotrophic sulfur oxidizer as generally believed, and the bacterium could grow well at a minimum rate by the addition of the organic matter tested.

The previous study (MATSUYAMA, 1988) showed that without an external addition of organic matter *Macromonas* sp. could grow in the bacterial plate samples as long as environmental H_2S was low enough to limit the growth of *Chromatium* sp. What factor(s) favor the bacterial growth at the H_2S boundary of Lake Kaiike?

Fig. 3. Cell numbers of *Macromonas* sp., *Chromatium* sp., *Lamprocystis roseopersicina* and H₂S concentration in the bacterial plate sample (8 August 1990) incubated in 65-ml glass bottles for 18 days daily added with different amounts of the medium of DUBININA and GRABOVICH (1984) and H₂S. DG medium 0, 1, 2, 3, 4 and 5 indicates the daily addition of 0, 0.05, 0.1, 0.3, 0.5 and 1.0 ml of the medium per bottle. H₂S 0, 1, 2 and 3 indicates the daily addition of 0, 0.5, 1.0 and 2.0 ml of H₂S solution per bottle. Bottles without H₂S addition were capped with porous silicon rubber stoppers in order to keep an aerobic condition. All the bottles added with H₂S exhibited a chocolate-brown color due to a dense blooming of an aggregate-forming bacterium shown in Figure 1F.

A bacterial plate sample collected on 8 August 1990 was immediately incubated in the continuous culture system shown in the previous study (MATSUYAMA, 1987b). Inorganic medium of PFENNIG (1965) for marine strains was supplied with a peristaltic pump into the chamber (340 ml) which was filled with the sample. H₂S was added to give a rate of 2 mg S · l⁻¹ · d⁻¹. The chamber was kept in a water bath at 25°C and lighted at 200 lux with an incandescent lamp.

Figure 4A shows the time change of the cell number of *Macromonas* sp. The number of *Chromatium* sp. remained below 1 × 10⁵ cells · ml⁻¹ throughout the incubation. In spite of the

large biomass in an initial sample, *Macromonas* sp. decreased in number faster than the dilution rate showing the growth rate of -0.004 · hr⁻¹. But the bacterial response was different when *Chromatium* sp. was abundant in the sample. Figure 4B shows the time changes of the cell numbers of the two species in the continuous culture at the same condition. The chamber was filled with sample collected on 12 December 1987, and H₂S was added at a reduced rate of 0.2 mg S · l⁻¹ · d⁻¹. Sulfur globules rapidly disappeared from *Chromatium* sp., and its number decreased showing that H₂S was already limiting. (The growth rate of *Chromatium* sp. was estimated as -0.003 · hr⁻¹). But *Macro-*

Fig. 4. Time changes of the cell numbers of *Macromonas* sp. (a) and *Chromatium* sp. (b) in the bacterial plate samples continuously supplied with inorganic medium of PFENNIG (1965). A, C: the sample collected on 8 August 1990. B: the sample collected on 12 December 1987. The straight line indicates the dilution rate, $0.010 \cdot \text{hr}^{-1}$ (A), $0.004 \cdot \text{hr}^{-1}$ (B) and $0.011 \cdot \text{hr}^{-1}$ (C). When the *Chromatium*-deficient sample was added with H_2S at a rate of $2 \text{ mgS} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ (A), *Macromonas* sp. decreased the number. When the sample contained a considerable number of *Chromatium* sp. (B), or the *Chromatium*-deficient sample was added with laboratory-cultured *Chromatium* sp. (C), *Macromonas* sp. showed a positive growth. The growth rate of *Macromonas* sp. derived from the method of least square was $-0.004 \cdot \text{hr}^{-1}$ (A) and $0.006 \cdot \text{hr}^{-1}$ (B, C).

monas sp. gradually increased at a rate of $0.006 \cdot \text{hr}^{-1}$ (mean doubling time=5 days). A positive growth rate of *Macromonas* sp. was also obtained in the continuous culture of the bacterial plate sample collected on 8 August 1990 (Fig. 4C): H_2S -deficient inorganic medium of PFENNIG (1965) was supplied, and two ml of laboratory cultured *Chromatium* sp. (1.3×10^8 cells) was added daily to the chamber.

From these laboratory incubations of the bacterial plate samples it may be assumed that *Macromonas* sp. could grow by taking some organic substrates which became available as long as *Chromatium* sp. existed in considerable number and the growth of *Chromatium* sp. was H_2S -limited. A large number of *Macromonas* sp. found at the H_2S boundary in October 1989 and August 1990 (Fig. 2) may be a transient phenomenon caused by the foregoing partial breakdown of the dense population of *Chromatium* sp. due to an elevated temperature.

The large pearl white inclusions of *Macromonas* sp. are composed of CaCO_3 (MATSUYAMA, unpubl.). The inclusions were readily dissolved with diluted CH_3COOH as observed in *Achromatium oxaliferum* (DE BOER *et al.*, 1971), or gradually disappeared from the cells when H_2S accumulated in the culture (MATSUYAMA, 1988). After disappearance of the inclusions, several small globules, probably composed of sulfur (SKUJA, 1956; LA RIVIERE and SCHMIDT 1981), became apparent in the cells. *Macromonas* sp. is suggested to possess the capacity to oxidize H_2S for the protective detoxicifying H_2S oxidation. When the inclusion-deprived cells due to excess H_2S were exposed to air, the cells began to form the inclusions again within a short period, and the inclusion-deposited cells showed motility (MATSUYAMA, 1988). Bacterial capacity to accumulate such inclusions is assumed to be one of the essential activities to maintain life, and it may be a cellular device to exist at the H_2S boundary, where *Macromonas* sp. meets some organic substrates, probably supplied from coexisting *Chromatium* sp. It is noteworthy that *Chromatium* sp. readily consumed the sulfur globules when the bacterium was exposed to

more light, or as soon a H_2S supply was interrupted. But such sulfur-free cells of *Chromatium* sp. were hardly encountered at the site of the H_2S boundary of Lake Kaiike. Sulfur-free cells seemed to be rapidly eliminated for subsequent decomposition. Studies are under way to examine the above assumptions.

摘 要

貝池 H_2S 層上端に密集する *Macromonas* sp. の集積培養

2 種類の大型の細菌, *Macromonas* sp. と *Chromatium* sp. が貝池 H_2S 層上端に密集している (バクテリアプレート)。*Macromonas* sp. の生長を, バクテリアプレート試料を実験室で種々の条件下で培養し, 検討した。本細菌の集積培養は酢酸ナトリウム, カザミノ酸, および酵母エキスを含む DUBININA and GRABOVICH (1984) の培地で可能であった。 H_2S は本細菌の生長に阻害的であって, 本細菌は好氣的, もしくは微好氣的従属栄養生物であると推察された。これら有機物をバクテリアプレート試料に添加しなくても, *Chromatium* sp. が顕著に存在し, しかも *Chromatium* sp. の生長が H_2S により制限されている限り, 本細菌は生長した。

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- (著者: 松山通郎, 長崎大学水産学部, 〒852 長崎市文教町1-14; Michiro MATSUYAMA, Faculty of Fisheries, Nagasaki University, Bunkyo-machi 1-14, Nagasaki 852).

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