Note

Buoyant Density of *Chromatium* sp.: Its Effect on the Blooming at an Upper Boundary of the H₂S Layer in Lake Kaiike

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Abstract

Two kinds of motile large-celled bacteria, *Chromatium* sp. and *Macromonas* sp., form steady-state dense populations at the upper boundary of the H₂S layer in Lake Kaiike. Buoyant density of *Chromatium* sp. estimated as about 1.26 g·cm⁻³ was considerably higher than that of the habitat. Cells of *Chromatium* sp. killed with formalin settled at a rate of about 0.8 cm·hr⁻¹. Living cells rapidly moved towards an upper light source when the number was lower than 10⁶ cells·ml⁻¹. However, when the number reached to a level of 2-5×10⁶ cells·ml⁻¹, further upward movement was not observed. Number of *Chromatium* sp. at the upper boundary of the H₂S layer, 10⁵-10⁶ cells·ml⁻¹, was considered to approximate the maximum level attainable at the boundary: above this number the bacterium seems unable to find swimming space sufficient to respond to tactic stimulus due to the excess crowding.

Key words: *Chromatium* sp., buoyant density, blooming, Lake Kaiike

At the upper boundary of the H₂S layer in Lake Kaiike, two motile large-celled bacteria, *Chromatium* sp., probably a new species of the Chromatiaceae family (MATSUYAMA, 1987a), and *Macromonas* sp., form steady-state dense populations (bacterial plate) throughout a year. The two species were characterized by their intracellular bodies: the former by many sulfur globules and the latter by 2 or 3 large pearl white refractile inclusions composed of CaCO₃ (MATSUYAMA, unpubl.). The ecological role of the deposition of sulfur globules in the former was mentioned earlier: the bacterium needs to store H₂S as sulfur globules inside the cells to have the advantage over other H₂S-competitors such as *Macromonas* sp. (MATSUYAMA, 1990).

Generally, deposition of sulfur globules in the phototrophic bacterial cells increases the buoyant density (MAS *et al.*, 1985; MAS and GEMERDEN, 1987). GUERRERO *et al.* (1984) showed that buoyant densities of *Chromatium*

warmingii and *Chr. vinosum* increased with deposition of sulfur globules from 1.071 to 1.108 g·cm⁻³ and 1.096 to 1.160 g·cm⁻³, respectively.

The cell number of *Chromatium* sp. at the upper boundary of the H₂S layer in Lake Kaiike is 10⁵-10⁶ cells·ml⁻¹ throughout a year, which is comparable to the maximum numbers of other Chromatiaceae species reported from various natural unpolluted waters (KUSNEZOW, 1959; GORLENKO and KUSNEZOW, 1972; COHEN *et al.*, 1977; GORLENKO *et al.*, 1978; BERGSTEIN *et al.*, 1979; ABELLA *et al.*, 1980; GUERRERO *et al.*, 1985). This fact suggests that the cell number of *Chromatium* sp. in Lake Kaiike approximates the maximum level attainable in natural waters or at least in Lake Kaiike. This study aims to consider the effect of buoyant density of *Chromatium* sp. on its blooming in Lake Kaiike.

Figure 1 shows the vertical profiles of the two bacterial species at the center of Lake Kaiike on 7 May 1988 together with some environmental factors. Apparatus and methods used for

Fig. 1. Vertical profiles of *Chromatium* sp. (A) and *Macromonas* sp. (B) in Lake Kaiike on 7 May 1988 together with some environmental factors.

this study were already described (MATSUYAMA, 1982). The two species, which were all filled with the intracellular bodies, appeared at the upper boundary of the H_2S layer (5.5 m depth) (Fig. 2), and both showed their maximum numbers at or just below this depth. Then, their numbers rapidly decreased with depth. In this period, the bacterial peaks were found in the lower part of the pycnocline at which σ_t was 22.0 ($\sigma_t = (\rho_{s,t} - 1.000) \times 1000$, where $\rho_{s,t}$ is density of water determined by chlorinity and temperature). However, the bacterial peaks did not always coincide with the pycnocline. On 15 December 1987, *Chromatium* sp. was most abundant at 6.5 m depth (1.5×10^5 cells $\cdot$ ml $^{-1}$), 3 m below the lower limit of the pycnocline, and σ_t at the bacterial peak was 23.7 (MATSUYAMA, unpubl.). The depth showing the peaks of two bacterial species, which usually overlapped, was determined by the profile of incident light: the peaks were regularly found just below the algal compensation point where surface light was reduced below a 10% level (MATSUYAMA, 1985).

Bacterial plate sample collected from 5.5 m depth on 25 October 1988 was filled in 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 equally distributed to form a bacterial suspension. Each 0.5 ml of the suspension (*Chromatium* sp.: 1.4×10^8 cells) was gently poured on the top surface of 50-ml sucrose solutions having different densities from 1.02 to 1.33 g $\cdot$ cm $^{-3}$, and centrifuged again. Microscopic observation showed that there was no significant change of the cellular volume due to osmotic shock. Figure 3 shows the bacterial numbers deposited on the bottom or floating in the surface layer in each sucrose solution: with increase of the density *Chromatium* sp. began to float into the overlying solution and to form a sharply defined purplish red band at the subsurface layer. This bacterial floatation became marked above the density of 1.26 g $\cdot$ cm $^{-3}$, suggesting that the mean buoyant density of

Fig. 2. Microphotograph of the bacterial plate collected from 5.5 m depth on 25 October 1988. *Chromatium* sp. (A) and *Macromonas* sp. (B) were dominant. The two species were each filled with many sulfur globules and 2 or 3 large pearl white inclusions.

Chromatium sp. was about $1.26 \text{ g}\cdot\text{cm}^{-3}$. This suggestion was also shown with the pure culture of *Chromatium* sp., and it was well consistent with that of *Chromatium vinosum* ($1.25 \text{ g}\cdot\text{cm}^{-3}$) reported by MAS *et al.* (1985). It is worth noting that coexisting *Macromonas* sp. showed a more increased buoyant density between $1.26 - 1.30 \text{ g}\cdot\text{cm}^{-3}$ (MATSUYAMA, unpubl.), probably due to the large CaCO_3 inclusions (DE BOER *et al.*, 1971).

Sinking property of *Chromatium* sp. killed with formalin was examined. Pure culture of *Chromatium* sp., which was grown in the medium for marine strains of PFENNIG (1965), was added with a few drops of formalin to kill the cells, and equally dispersed into the glass tubes (25 cm tall, 1.1 cm i. d.). The tubes were tightly capped with silicon rubber stoppers at both ends and stood upright in a water bath at 25°C . After 1, 2, 6, 9, 13 and 18 hr, these tubes were opened for examination. Two ml of sample (2 cm long in the tube) was successively taken up with a pipette from the top layer to the lowest one just above the bottom with care not to disturb the underlying bacterial profile. In order to prevent thermal convection, which

readily occurred with a slight temperature shock, most of the tube was kept in a water bath during the sampling. Figure 4 shows the profile of the bacterial cells at different periods. Immediately after beginning the experiment, the cells began to sink. As the cells were perturbed by Brownian motion, the upper boundary of the falling cells was taken as the layer in which the initial cell number ($1.2 \times 10^6 \text{ cells}\cdot\text{ml}^{-1}$) was reduced by half. Time change in the boundary thus estimated indicated that the cells sank with a constant speed of about $0.8 \text{ cm}\cdot\text{hr}^{-1}$.

Earlier microscopic observation of the living cells of *Chromatium* sp. showed that the cell moved back and forth in random directions with a maximum speed of $20 \mu\text{m}\cdot\text{sec}^{-1}$ (MATSUYAMA, 1987a). But the bacterium seems to approach the tactic stimulus by the modulation of frequency of change in swimming direction, as SISTROM (1978) pointed out.

Culture of *Chromatium* sp. was centrifuged (15 min, $1000 \times g$). Each one ml of the bacterial suspension (2.8×10^8 cells) was gently injected into the bottom of the glass tubes which were previously filled with the marine medium

Fig. 3. Numbers of deposited cells of *Chromatium* sp. (dotted column) and floating at subsurface layer (striped column) in sucrose solutions having different densities after centrifugation (15 min, $1000\times g$). Above the density of $1.26\text{ g}\cdot\text{cm}^{-3}$, the number of the deposited cells rapidly decreased, and the bacterium formed a sharply defined purplish red band at subsurface layer. Left: the bacterial plate sample (total number of *Chromatium* sp.: 1.4×10^8 cells). Right: pure culture of *Chromatium* sp. (total number: 3.0×10^8 cells). Cells forming a subsurface band were expressed as number $\cdot\text{ml}^{-1}$. Number of floating cells could be obtained by subtraction of the number of deposited cells from total one.

of PFENNIG (1965). The tubes were tightly capped and stood upright in a water bath at 25°C . Light was provided with incandescent lamps placed above the tubes to give the intensity of 100 lux at the top of the tubes to cause the bacterial upward movement. Under such a dim light the bacterial growth during the experiment was negligible (MATSUYAMA, 1987b).

Figure 5 shows the bacterial profile at different periods. The cells killed with formalin settled rapidly on the bottom, whereas the living cells moved upwards. At 4 hr later, the number in the top layer was 1.7×10^5 cells $\cdot\text{ml}^{-1}$.

But further upward movement of the cells became poor. From 11 hr until the end of the experiment (47 hr), almost no change was observed in the profile: the number in the top layer was $2\text{--}5\times 10^5$ cells $\cdot\text{ml}^{-1}$, and it increased almost exponentially with depth to show the number of $0.7\text{--}1.6\times 10^8$ cells $\cdot\text{ml}^{-1}$ just above the bottom of the tubes. A similar bacterial profile was also formed in the glass tubes which were stood upright for 24 to 48 hr after the cells were equally distributed (not shown here). Figure 5 indicated that the upward movement of the bacterial cells was hindered due to the

Fig. 4. Sinking property of pure culture of *Chromatium* sp. in the glass tubes (25 cm tall, 1.1 cm i. d.) filled with the marine medium of PFENNIG (1965) (NaCl and MgSO_4 contents were 25 g and $3.8 \text{ g} \cdot \text{l}^{-1}$, respectively). Density of the medium was $1.020 \text{ g} \cdot \text{cm}^{-3}$ at 25°C . The cells were previously killed with formalin and equally dispersed in the tubes (total number: 2.4×10^7 cells). All the bacterial cells were filled with sulfur globules. Numeral indicates the time (hr).

Fig. 5. Moving property of pure culture of *Chromatium* sp. in the glass tubes which were previously filled with the marine medium of PFENNIG (1965). One ml of bacterial suspension (2.8×10^8 cells) was injected into the bottom of tubes. Light was provided with incandescent lamps placed above to give the intensity of 100 lux at the top of tubes. Dotted line: the cells killed with formalin. Numeral indicates the time (hr).

excess crowding when the number reached the level of $2\text{--}5 \times 10^6$ cells $\cdot\text{ml}^{-1}$. If the bacterial cells were supposed to distribute equally in the tube, the number would be expected to reach 1.2×10^7 cells $\cdot\text{ml}^{-1}$. Microscopic observation showed that even the cells deposited on the bottom were motile, but the space available for their free-swimming activity was greatly limited: the cells only moved just around their vicinity. This is also seen in the batch culture of *Chromatium* sp. in a large vessel: the cells, which has previously formed a homogeneous suspension in the whole vessel, began to sink to form the sediments when the number exceeded a level of 5×10^6 cells $\cdot\text{ml}^{-1}$, although the cells deposited on the bottom were all motile (MATSUYAMA, 1987a). Apparently, both the increase in the buoyant density and the excess crowding resulted in the bottom-dense bacterial profile in the tubes (Fig. 5), which was markedly different from the top-dense profile in the H_2S layer of Lake Kaiike (Fig. 1).

As seen in Figure 1, the water around the bacterial habitat was very stable due to a large density gradient, and the water was far lighter than the bacterial body. To stay precisely at the upper boundary of the H_2S layer, where *Chromatium* sp. meets light, the bacterium must depend on its swimming activity. *Macromonas* sp. was also abundant at the boundary ($10^5\text{--}10^7$ cells $\cdot\text{ml}^{-1}$). Not only interspecies, but also intraspecies blocking would operate when there is an increase in the number of *Chromatium* sp. beyond the present level. The cell number of *Chromatium* sp. at the upper boundary of the H_2S layer could be considered to approximate the maximum level attainable at the boundary: above that number the bacterium seems unable to find swimming space sufficient to respond the tactic stimulus.

It is probable that *Chromatium* sp. must expend much energy for swimming activity. Previous studies (MATSUYAMA, 1986, 1987b, 1988) showed that the bacterium must depress its photosynthetic activity to a minimum level in spite of the large biomass at the site of the upper boundary of the H_2S layer. The bacterial burden involved in keeping its heavy body

at the boundary by its swimming activity may be a factor in such a low photosynthetic activity. Quantitative considerations of the bacterial energetics between growth and swimming activity are necessary.

摘 要

Chromatium sp. の浮遊密度：その貝池 H_2S 層上端でのブルーム形成におよぼす影響

運動性を持つ大型の2種類の細菌、*Chromatium* sp. と *Macromonas* sp., は貝池 H_2S 層上端に安定した濃密群集を形成している。*Chromatium* sp. の浮遊密度は約 $1.26 \text{ g} \cdot \text{cm}^{-3}$ と推定され、その生息環境の密度に比較し著しく高かった。ホルマリンで殺した *Chromatium* sp. の細胞は約 $0.8 \text{ cm} \cdot \text{hr}^{-1}$ の速度で降下した。生きている細胞は濃度が 10^6 細胞 $\cdot\text{ml}^{-1}$ 以下の時上方の光源に向け急速に移動した。しかし、濃度が $2\text{--}5 \times 10^6$ 細胞 $\cdot\text{ml}^{-1}$ に達すると上方への移動は見られなくなった。 H_2S 層上端での *Chromatium* sp. の濃度、 $10^5\text{--}10^6$ 細胞 $\cdot\text{ml}^{-1}$ はそこで達し得る最高出現量にほぼ等しいと考えられ、その濃度以上では細菌は過密により目標とする環境刺激に充分応答できる自由遊泳距離を確保できなくなると思われる。

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