Note

Frequency of Dividing Cells of *Chromatium* sp. Blooming in Lake Kaiike as an Estimate of Growth Rate

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Abstract

*Chromatium* sp. blooming at an upper boundary of the H₂S layer of Lake Kaiike was cultured in the medium of PFENNIG (1965) under a variety of light intensities. Specific growth rate of the bacterium (Y) could be approximated by frequency of the dividing cells (X) as follows:

\[ Y = 0.098e^{3.8X} \quad (r = 0.609) \]

indicating that measurement of frequency of the dividing cells was very simplified method adequate to estimating the growth rate. *In situ* specific growth rate of the bacterium could be estimated as about 0.17·day⁻¹, which is only about twice the maintenance rate constant obtained in the pure culture. Bacterial ability to depress growth capacity to such a low level in response to environmental limitations is considered one of the factors causing the bloom in Lake Kaiike.

Key words: *Chromatium* sp., frequency of dividing cells, growth rate, Lake Kaiike

Two large-celled bacterial species, *Chromatium* sp. and *Macromonas* sp., bloom stably at an upper boundary of the H₂S layer of Lake Kaiike throughout the year to form a purplish red bacterial plate. Both species are characterized by the presence of intracellular bodies: *Chromatium* sp. by numerous sulfur globules; and *Macromonas* sp. usually by two large pearly white inclusions consisting of CaCO₃. These bodies seem disadvantageous to their planktonic lives, since they significantly increase cellular buoyant densities to or above 1.26 g·cm⁻³ (MATSUYAMA, 1991). Without these bodies, however, neither species could maintain cell-integrity. They are thought to be cellular adaptations aiding survival at an upper boundary of the H₂S layer (MATSUYAMA, 1990, 1992). Increased cellular buoyant densities seem to effectively eliminate bacterial cells in the inactive state from that an upper boundary.

*In situ* measurement of N₂ fixation rate shows that *Chromatium* sp. grows at a very low rate, although the bacterial number equals that attainable in natural waters (~10⁶ cells·ml⁻¹) (MATSUYAMA, 1986, 1988), and the bacterial cells in a dividing state are abundant. The present study aims to estimate *in situ* growth rate of the bacterium based on an empirical relationship between frequency of the dividing cells and growth rate obtained from the pure cultures.

Figure 1 is a microphotograph showing a bacterial plate sample of Lake Kaiike collected from a depth of 5.3 m on 10 April 1990 (salinity: 33.10 ‰, temperature: 19.9°C, H₂S: 5.7mgS·l⁻¹). The sample was collected in a 1-l sterile glass bottle and transferred to the laboratory under cool, dark conditions. Microscopic observations were performed within 48 hr after collection. Number of both species were maximum at that 5.3 m depth (*Chromatium* sp.: 1.5×10⁶ cells·ml⁻¹, *Macromonas* sp.: 1.1×10⁶ cells·ml⁻¹).
Fig. 1. Microphotograph of bacterial plate sample of Lake Kaiike collected from 5.3 m depth on 10 April 1990. Two large-celled bacterial species, *Chromatium* sp. containing numerous sulfur globules and *Macromonas* sp. having two pearly white inclusions, are dominant. Arrow indicates a dividing cell of *Chromatium* sp.

It is noteworthy that I found a significant proportion of *Chromatium* sp. cells showing a constriction of the cell walls, but no clear separation between daughter cells. In this study such bacterial cells are referred to as dividing cells. To measure frequency of the dividing bacterial cells (FDC) in the pure cultures, I counted ten microscopic fields (magnification: ×600) each of which had to contain more than 20 bacterial cells. To measure FDC in the bacterial plate sample (recorded on several microphotographs at each sampling), a total of 200 cells were counted. FDC was obtained enumerating both total and dividing bacterial cells.

Table 1 summarizes FDC values of *Chromatium* sp. in the bacterial plate sample collected on different days. FDC is expected to be positively related to the bacterial growth as shown in other aquatic bacteria (HAGSTROM et al., 1979; NEWELL and CHRISTIAN, 1981). Purely isolated *Chromatium* sp. from Lake Kaiike was cultured in the inorganic medium of PFENNIG (1965). Inoculated medium in a series of 160-ml bottles was incubated at 25°C under varying light intensities provided by an incandescent lamp. Light intensity was altered by wrapping the bottles in layers of black nylon net.

Figure 2 shows the bacterial cultures. Every two days a 1.0 ml subsample was removed with a sterile PASTEUR pipette and dropped on a slide glass for measurement of FDC or on a THOMA hemacytometer for bacterial counting. The bottles were supplied with a freshly prepared

<table>
<thead>
<tr>
<th>Date</th>
<th>Depth (m)</th>
<th>FDC</th>
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<tbody>
<tr>
<td>26 Apr. 1984</td>
<td>5.5</td>
<td>0.20</td>
</tr>
<tr>
<td>1 Nov. 1984</td>
<td>5.0</td>
<td>0.18</td>
</tr>
<tr>
<td>27 July 1987</td>
<td>5.5</td>
<td>0.11</td>
</tr>
<tr>
<td>15 Dec. 1987</td>
<td>6.5</td>
<td>0.11</td>
</tr>
<tr>
<td>17 May 1989</td>
<td>5.0</td>
<td>0.08</td>
</tr>
<tr>
<td>10 Apr. 1990</td>
<td>5.3</td>
<td>0.13</td>
</tr>
<tr>
<td>4 Apr. 1991</td>
<td>5.0</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Fig. 2. Growth of *Chromatium* sp. in the medium of PFENNIG (1965) under varying light intensities. Numerals within frame indicate light intensity (lux).
medium of PFENNIG (1965) to continue the culture. Specific growth rate was obtained using the equation:

\[ N_{t+2} = N_t e^{2Y} \]

where \( Y \) was specific growth rate (d\(^{-1}\)), \( N_t \) and \( N_{t+2} \) were bacterial numbers at any time \( t \) and at two days after \( t \).

Figure 3 shows a relationship between FDC at time \( t \) (X) and a specific growth rate for \( t \) and \( t+2 \) (Y) during the logarithmic growth phase under varying light intensities. There is an empirical relationship expressed as

\[ Y = 0.098e^{3.8X} \quad (r=0.609). \]

Measurement of the FDC is considered to be a very simplified (no incubation) method which is nevertheless adequate to estimate the growth rate as far as the present bacterium is concerned.

If an FDC value simply averaged for seven periods shown in Table 1 (0.15) is assumed to be representative of the blooming Chromatium sp., its mean specific growth rate could be estimated as 0.17•d\(^{-1}\) (mean doubling time: 4 days). That is low compared with rates of aquatic bacteria estimated by similar methods:

<table>
<thead>
<tr>
<th>In situ</th>
<th>Specific growth</th>
<th>Reference</th>
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<tr>
<td>FDC (%)</td>
<td>rate (d(^{-1}))</td>
<td></td>
</tr>
<tr>
<td>0.6-6.0</td>
<td>0.17-1.7</td>
<td>HAGSTRÖM et al. (1979)</td>
</tr>
<tr>
<td>2.5-9.8</td>
<td>0.24-3.1</td>
<td>NEWELL and CHRISTIAN (1981)</td>
</tr>
<tr>
<td>8-26</td>
<td>0.13-0.26</td>
<td>This study</td>
</tr>
</tbody>
</table>

This estimation, however, did not contradict that deduced from in situ measurement of bacterial N\(_2\) fixation (MATSUYAMA, 1986). In situ growth rate of the bacterium thus estimated was only twice the maintenance rate constant, whereas specific growth rate was 0.07 • d\(^{-1}\) (MATSUYAMA, 1987b). Most bacterial cells are thought to grow very slowly. The need of bacterium to depress growth capacity to such a low level is primarily due to two limiting environmental factors, light and H\(_2\)S. Both factors are soon rendered inoperative once they enter the bacterial population. Therefore, in situ growth rate of the bacterium (and frequency of the dividing cells) may be viewed as being in a state of dynamic tension between the bacterial capacity to multiply and countervailing environmental limitations which become more severe as the bacterium increase in number. It is noteworthy that many bacterial cells are found in a dividing state irrespective of such a low growth rate. Further studies are necessary to more fully explain bacterial dynamics resulting in the steady-state bloom in Lake Kaiike.

Fig. 3. Relationship between frequency of dividing cells of Chromatium sp. (FDC) (X) and specific growth rate (Y) obtained from pure cultures under varying light intensities (○: 880 lux, ●: 500 lux, △: 320 lux, ▲: 200 lux, +: 130 lux). Regression line was drawn using least-squares method.

予想

貝池でブルームを形成している Chromatium sp. の生長速度推定法としての分裂細胞出現率

貝池H\(_2\)S層上端でブルームを形成している Chromatium sp. を PFENNIG (1965) の培地で異なる照度下培養した。細菌の比生長速度（Y）は分裂細胞出現率（X）によって次のように近似され、

\[ Y = 0.098e^{3.8X} \quad (r=0.609) \]

分裂細胞出現率の測定が生長速度を推定する上で有効できわめて簡便な方法であると考えられた。
MATSUYAMA

細菌の現場での比生長速度は約 0.17・日^{-1}と推定され、純粋培養で求められる値を維持する上で必要最小の比生長速度の約2倍にしか相当しない。細菌が環境制限に呼応し、生長能力をそのような低いレベルまで抑制できることは貝池でのブルーム形成を可能とする要因の一つと考えられる。

References


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