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Disturbance of *Chromatium* Population at Mid-depth of Lake Kaiike

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Two large-celled bacterial species, *Chromatium* sp. and *Macromonas* sp., densely populate the mid-depth of Lake Kaiike, a small lagoon on Kamikoshiki Island, turning the water a purplish red (bacterial plate). In recent years, the *Chromatium* population has showed large seasonal fluctuations; maximum cell numbers in the order of 10^6 cells ml^{-1} have dropped to 10^3 cells ml^{-1}. To examine the factor(s) disturbing the population, the profiles of two bacterial species as well as environmental factors were examined during the period June 1998 to Oct. 2001. Solar heating to raising the water temperature above 30°C as well as a proliferation of *Macromonas* sp. were found to have disturbed the *Chromatium* population.

Key words: *Chromatium* population, disturbance, solar heating, *Macromonas* sp., Lake Kaiike

Lake Kaiike on Kamikoshiki Island, Kagoshima Prefecture (surface: 0.15 km^2, max. depth: 11.6 m) stratifies due to an external intrusion of seawater through a gravel bar and due to covering by surface run-off. From spring to autumn, the temperature in the transition zone between the less saline layer and seawater layer (lying at a depth of 1–4 m) rose; reaching 39°C at 3 m on 26 Aug. 1976, 10 and 20°C higher than the temperatures of the upper and lower layers, respectively. The lake is well protected from wind work not only due to the two-layered system, but also due to the thermocline. Water below a depth of 4–5 m always contains a considerable amount of H_2S (~51 mg S l^{-1}). Two large-celled bacterial species, *Chromatium* sp. (3–4×3–6 μm), having many sulfur globules inside cells (probably a new species of the Chromatiaceae family) and *Macromonas* sp. (2–3×4–8 μm), having 1~3 large, pearl-white $CaCO_3$ inclusions, swarm at the upper boundary of the H_2S layer to color the water a purplish red (bacterial plate). Maximum cell numbers for these species were in the order of 10^6 cells ml^{-1}. The buoyant density of these cells is very high (~1.27 g cm^{-3}). The swarming at the upper boundary of the H_2S layer owe is due to swimming activity.

In recent years, the *Chromatium* population (as well as *Macromonas* sp.) has shown large seasonal fluctuations: the maximum cell number of *Chromatium* sp. changed from approximately 10^6 cells ml^{-1} in spring to 10^3–10^4 cells ml^{-1} during summer to early autumn. Frequently, bacterial coloring of the upper boundary of the H_2S layer, where *Chromatium* sp. usually exhibits maximum abundance, disappeared, and sulfur-deficient *Chromatium* cells, which were characteristics of the cells cultured under suboptimal

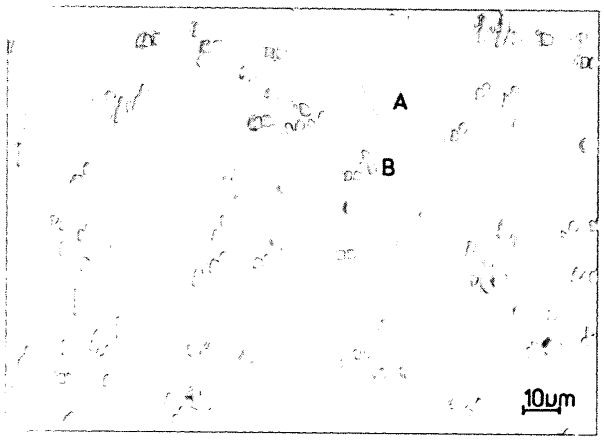

Fig. 1. Microphotograph of the bacterial plate collected from a depth of 5.5 m depth in Lake Kaiike on 23 Sept. 1998. *Chromatium* sp. (A) and *Macromonas* sp. (B) were dominant (3.5×10^5 cells and 1.1×10^6 cells ml^{-1}, respectively). *Chromatium* cells without sulfur globules were abundant.

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light conditions, appeared (Fig. 1). The present study aims to examine the factors which disturb the *Chromatium* population in the lake.

Materials and Methods

Observations were made at a central point of Lake Kaiike for 3 years from June 1998 to Oct. 2001. Water samples were collected with a 3 l sampler (Rigo Sampler, B Type, Rigosha Ltd.) at 1 m intervals from the surface down to a depth of 10 m. In the layer between 3 and 6 m, samples were taken at 0.5 m intervals. Temperature was measured with a mercury thermometer immediately after lake water was collected. Salinity was measured with an inductive salinometer (Tsurumi Digital Salinometer, Type E-202, Tsurumi Ltd.). Photosynthetically available radiation (PAR) was measured using a quantum sensor (LI-Cor Underwater Radiation Sensor, Type SA, LI-Cor Inc. USA). For the measurement of dissolved O_2, the Winkler method was applied. For the determination of H_2S, the titrimetric method was applied[1]. Samples for bacterial counting were kept in 50-ml bottles and neutralized formalin was added to give a final concentration of 1%. The samples were kept under cool, dark conditions to be transferred to the laboratory. Samples were centrifuged (20 min., 1,000×g) to concentrate planktonic microorganisms. Bacterial cells were enumerated with a Thoma hemocytometer. Standard deviation was 1.3×10^5 cells ml^{-1} for countings of *Chromatium* suspension giving a mean cell density of 19×10^5 cells ml^{-1} (n=16). Microscopic observations were made within 48 hrs after collection.

The effect of temperature on the flotation of isolated *Chromatium* cells was examined using a series of glass tubes (17.7 cm tall, 2.1 cm i.d.)[6,7]. A bacterial suspension reaching a logarithmic phase of growth in the inorganic medium of Pfennig[8] was mixed with a newly prepared suspension, and then distributed into glass tubes. The density of the medium was 1.02 g cm^{-3} at 25°C, being close to that of the original habitat. Tubes filled with inoculated medium were tightly closed and left in an incubator (Taitec Incubate Box, Type M-200F, Taitec Ltd.). The tubes were exposed to an incandescent lamp to give a PAR level of 3 μmol m^{-2} s^{-1} at the irradiated surface in a 12 hr light and dark cycle. Incubation was performed at temperatures of 10, 15, 20, 25, 28, 31, 33 and 35°C. Two tubes were taken for examination. Floating cells at different depths of the medium (0, 3, 6, 9, 12, 15 cm below the upper end) were carefully collected with a glass capillary so as not to disturb the bacterial profile for measurement of cell numbers.

Results and Discussion

Figure 2 shows the vertical profiles of *Chromatium* sp. and *Macromonas* sp. in Lake Kaiike together with environmental factors at the time when the subsurface zone heated above 30°C was expanding (from 26 May through 22 June 1999) or retreating (from 4 June through 23 Sept. 1998). The biomass values of the two bacterial species estimated from integrations of bacterial cells contained in a water column with a 1-cm^2 surface down to 10 m are given in Figure 3 with those at other times.

Throughout the experiments, H_2S always appeared at a depth of 4.5 m to 5.0 m, and increased with depth to show a maximum concentration ranging from 21 mg S l^{-1} (16 May

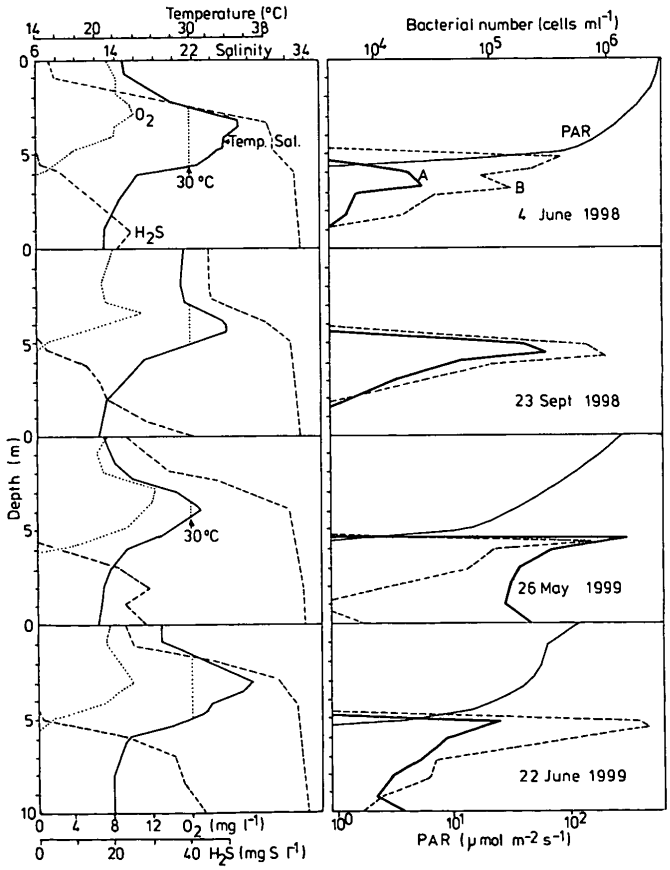

Fig. 2. Vertical profiles of *Chromatium* sp. (A) and *Macromonas* sp. (B) together with photosynthetically available radiation (PAR) in Lake Kaiike on 4 June and 23 Sept. 1998, and 26 May and 22 June 1999 (right column). Vertical profiles of environmental factors such as salinity (Sal.) and temperature (Temp.) are shown in the left column. PAR levels rapidly attenuated beyond a depth of 5 m due to the shading effect of microorganisms. The temperature of the upper boundary of the H_2S layer was 31.7°C on 4 June 1998 and 30.1°C on 23 June 1999. Profiles between depths of 3 m to 6 m were based on samplings taken at 0.5 m interval (Otherwise 1 m intervals).

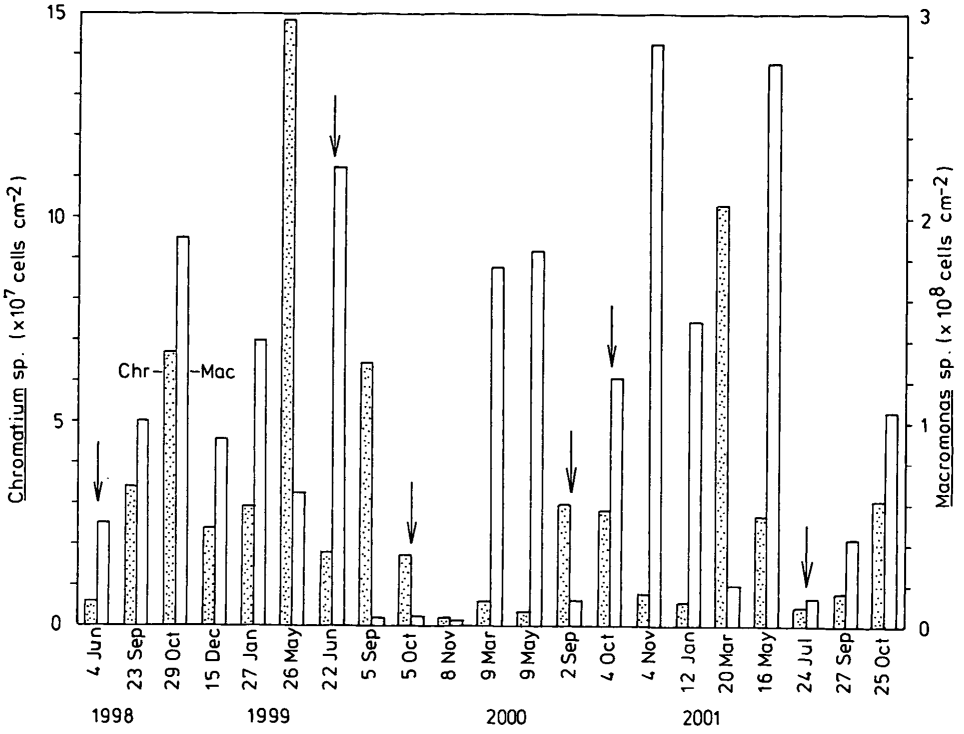

Fig. 3. Biomass of *Chromatium* sp. (dotted column) and *Macromonas* sp. (open column) in Lake Kaiike from June 1998 to Oct. 2001. Biomass was estimated from the integration of bacterial cell numbers in a water column from surface to 10 m deep (unit: cells cm^{-2}). Arrows indicate the time when the temperature of the upper boundary of the H_2S layer was >30°C. Typhoon 18 which struck Kamikoshiki Island on 24 Sept. 1999 was the most intense ever recorded in this region; maximum surface wind speed was >60 m s^{-1} at Nakakoshiki. But the deeper H_2S layer of Lake Kaiike remained in a steady-state, although the temperature of the upper boundary of the H_2S layer (5 m depth) rose from 29.3°C on 5 Sept. to 30.9°C on 5 Oct. 1999.

2001) to 51 mg S l^{-1} (5 Oct. 1999) just above the bottom.

PAR was rapidly reduced at the upper boundary of the H_2S layer as already observed. On 4 June 1998, a peak in the *Chromatium* population was observed at 6.5, being 1.5 m below the upper boundary of the H_2S layer, and PAR at the peak was far less than the minimum requirement for *Chromatium* cells to retain swimming activity[7]. On 23 Sept. 1998, the bacterial peak returned to the upper boundary of the H_2S layer, and the bacterium increased in biomass.

Temperature showed a pronounced maximum at a depth of 3–4 m during spring to autumn. Due to a large density gradient between the upper less saline layer and deeper seawater layer, incident solar radiation was effectively trapped in this transition zone ("solar lake"[2]). A maximum temperature of 38.4°C was recorded at 3.5 m on 5 Sept. 1999. Profiles of these environmental factors were similar to those reported previously[5,6].

The populations of *Chromatium* sp. and *Macromonas* sp. showed large seasonal changes (Figs. 2, 3). Generally, they were large in spring and autumn, and small in summer and winter. Cell numbers of *Chromatium* sp. peaked on 26 May 1999 (1.4×10^6 cells ml^{-1} at 5.5 m), and the biomass attained a level of 15×10^7 cells cm^{-2}, being close to the maximum ever recorded (17×10^7 cells cm^{-2} on 7 May 1988)[5]. Cell numbers were smallest on 8 Nov. 1999 (2×10^3 cells ml^{-1} at 6 m), and the biomass decreased to 2×10^6 cells cm^{-2}. It is worth noting that the biomass of *Chromatium* sp. was apt to fall when the upper boundary of the H_2S layer was heated above 30°C (as shown by a rapid decrease in *Chromatium* biomass from 26 May through 22 June 1999 in Fig. 2), and sometimes the bacterial peak shifted from the upper boundary of the H_2S layer down to a depth where the temperature fell just below 30°C as shown in Figure 2. When the upper boundary of the H_2S layer cooled below 30°C, *Chromatium* sp. increased in biomass again (as shown by the increase of *Chromatium* biomass from 4 June through 23 Sept. 1998 in Fig. 2) unless *Macromonas* sp. increased in number more rapidly. On 2 Sept. 2000, however, the biomass of *Chromatium* sp. was larger than previously recorded (9 May 2000) in spite of the high temperature of the upper boundary of the H_2S layer. At this time, the *Chromatium* peak was observed at 6.5 m, being 1.5 m lower than the upper boundary of the H_2S layer. The sudden decrease in *Macromonas* biomass during this period might favor the *Chromatium*

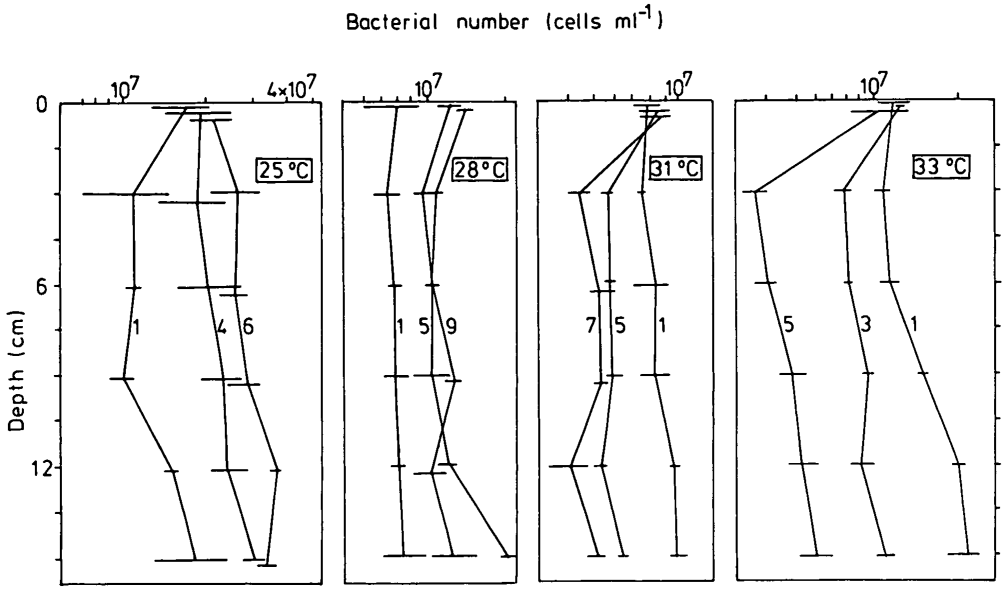

Fig. 4. Vertical profiles of *Chromatium* sp. incubated in glass tubes at temperatures between 25 and 33°C. Each incubation was performed in duplicate. Numbers indicate incubation time (day). Horizontal lines represent the range of measurements of bacterial cell numbers.

peak at a lower depth.

The Kaiike strain of *Chromatium* sp. grew fast at temperatures of 30–32°C (μ=1.0 day^{-1}) and showed a negative rate of growth (–0.11 day^{-1}) just above 33°C[4].

Figure 4 shows the changes with time in the vertical profiles of *Chromatium* density in the liquid medium between 25 and 33°C. The time change rate of floating cell number, k, was obtained from the following equation:

$$N_t=N_1e^{(t-1)k}$$

Where N_1 and N_t are floating cell numbers at 1 and t days after beginning incubation. Figure 5 shows k thus obtained including those at other temperatures. At temperatures between 15 and 25°C, numbers of floating cells consistently increased. But at 31°C, they began to decrease. At 35°C, the cells were lost as rapidly as those kept in continuous darkness[7]. It must be noted that even at the end of incubation at 35°C, the total number of cells in each tube (sum of floating and sedimented cells) was almost identical to the initial number, indicating that no significant cell growth or lysis occurred during the incubation, and some of the sedimented cells began to float again when they were incubated at 25°C.

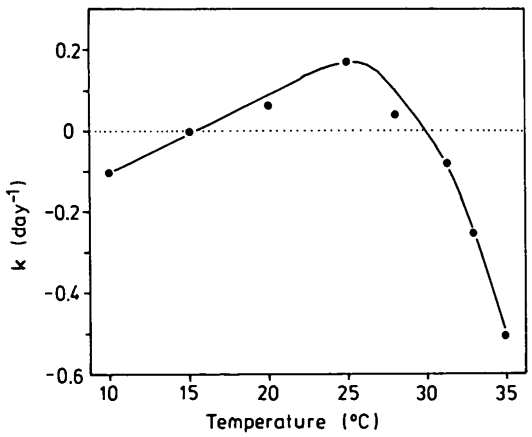

Fig. 5. Comparison of time change rate of the floating cells of *Chromatium* sp. (k), incubated at temperatures between 10 and 35°C. k was obtained from the change in floating cell numbers 1 day after the beginning of incubation and end of incubation.

Figure 6 shows the highest and lowest vertical profiles of water temperature observed at different depths of Lake Kaiike during two previous periods, Apr. 1976–Feb. 1977 (A) (n=6) and Apr. 1984–Jan. 1985 (B) (n=6)[3], and during the period studied here, June 1998–Oct. 2001 (C) (n=21). During the previous two periods, the temperature at a depth of 5 m changed from 10 to 30°C. While, during June 1998–Oct. 2001, the temperature at this depth frequently rose above 30°C. The marked decrease in temperature below the mid-depth in the winters of the previous periods (<14°C at a depth of 5 m), probably induced by partial mixing with upper cool water, would be a latent factor preventing the bacterial habitat from heating above 30°C in the next spring to autumn.

As shown previously[6,7], *Chromatium* sp. began to move upwards when the cells were exposed to a level PAR of 0.6 μmol m^{-2} s^{-1} (in the 12 hr light and dark cycle (25°C)), and the bacterium began to grow at a PAR above 1 μmol m^{-2} s^{-1}. However, when the bacterial suspension was placed at a re-

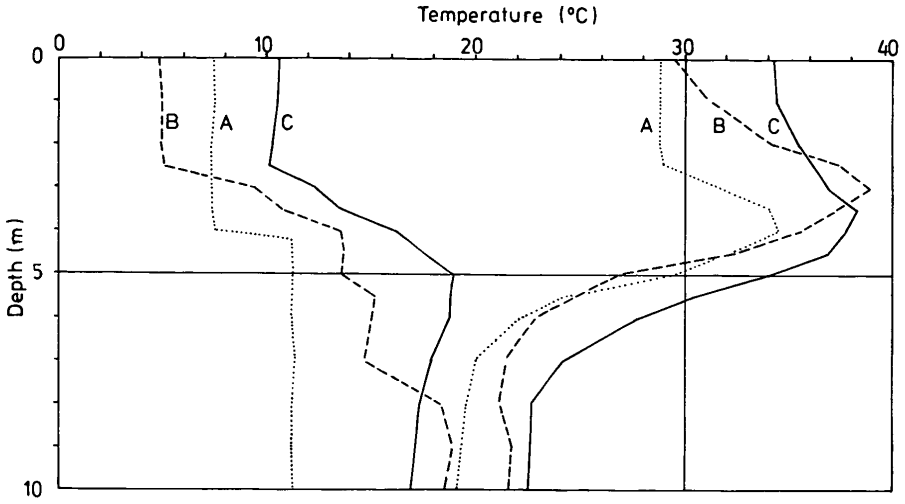

Fig. 6. Highest and lowest vertical profiles of water temperature observed at different depths in Lake Kaiike during Apr. 1976–Feb. 1977 (A) (n=6), Apr. 1984–Jan. 1985 (B) (n=6) and June 1998–Oct. 2001 (C) (n=21).

duced PAR level of 0.2 μmol m^{-2} s^{-1}, floating cells gradually disappeared. Bacterial cells once driven into the dark were suggested to move upwards towards light. This bacterial behavior seemed to result in the elimination of cells from the original habitat when the incident solar radiation heated the water above 30°C, and when incident light was rapidly extinguished by other microorganisms. The finding that the peak of *Chromatium* sp. occurred at 6.5 m on 4 June 1998 (Fig. 2), being 1.5 m deeper than the upper boundary of the H_2S layer, may be a result of the elimination of *Chromatium* cells from the upper heated section of the bacterial plate due to solar radiation as well as due to dense coverage by the *Macromonas* population. If the cooling of the lake water was insufficient in winter, and solar heating of the upper boundary of the H_2S layer above 30°C continued until mid-autumn (as in 1999, 2000), many *Chromatium* cells would be eliminated from the habitat.

In conclusion, the *Chromatium* population at the upper boundary of the H_2S layer was disturbed by solar heating as well as the proliferation of *Macromonas* sp. The dense population of *Chromatium* sp. in spring was in the order of 10^6 cells ml^{-1}, which would be the upper limit of occurrence of large-celled *Chromatium* species in natural unpolluted waters[9], and could be understood given the passing away of habitat- and biotic disturbances.

References

1) American Public Health Association. 1965. Standard methods for the examination of water and wastewater. 12th ed. APHA, N.Y.
2) Cohen Y., W.E. Krumbein, M. Goldberg and M. Shilo. 1977. Solar Lake (Sinai). Physical and chemical limnology. Limnol. Oceanogr. **22**: 597–608.
3) Matsyuama, M. 1985. Stratified condition of Lake Kaiike on Kamikoshiki Island with reference to inflow and outflow of seawater. Jpn. J. Limnol. **46**: 229–238 (in Japanese).
4) Matsuyama, M. 1987. A large phototrophic bacterium densely populating the O_2-H_2S interface of Lake Kaiike on Kamikoshiki Island, Southwest Japan. Acta Academiae Aboensis **47**: 29–43.
5) Matsuyama, M. 1991. Buoyant density of *Chromatium* sp.: Its effect on the blooming at an upper boundary of the H_2S layer in Lake Kaiike. Jpn. J. Limnol. **52**: 57–63.
6) Matsuyama, M. 1995. Upward movement of *Chromatium* sp. in the H_2S-layer of Lake Kaiike causing a bloom at its upper boundary. Jpn. J. Limnol. **56**: 205–209.
7) Matsuyama, M. 1996. Further comments on upward movement of *Chromatium* sp. in the H_2S layer of Lake Kaiike. Jpn. J. Limnol. **57**: 179–182.
8) Pfennig, N. 1965. Anreicherungskulturen für rote und grüne Scwefelbakterien. Zbl Bakt. 1. Abt. Orig. Suppl. **1**: 179–189, 503–505.
9) Gemerden, V. and J. Mas. 1995. Ecology of phototrophic sulfur bacteria. *In* Blankenship, R.E. (ed.), Anoxygenic photosynthetic bacteria. Kluver Academic.