Note
Low Growth Rate of a Large-celled Phototrophic Bacterium as a Factor to Form the Dense Population at the Dissolved O₂-H₂S Interface of Lake Kaiike

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Abstract

Light-limited growth was compared in two phototrophic bacteria, a large-celled one which was densely populating the dissolved O₂-H₂S interface of Lake Kaiike, and a Chromatium buderi strain DSM 176 obtained from the German Collection of Microorganism. C. buderi could grow fast at a high light intensity, but in a light-limited condition similar to that at the dissolved O₂-H₂S interface of Lake Kaiike C. buderi could not form a dense population. The intrinsic low growth rate of a large-celled phototrophic bacterium isolated from Lake Kaiike was considered to be its means of survival under light-limited conditions.

Key words: light-limited growth of a large-celled phototrophic bacterium, Lake Kaiike, comparison with Chromatium buderi.

Previous studies on a large-celled phototrophic bacterium isolated from the dissolved O₂-H₂S interface of Lake Kaiike, probably a new species of the family Chromatiaceae (MATSUYAMA, 1986, 1987a, b), suggested that the bacterium grew slowly even under optimal conditions, and the bacterium could maintain the dense population attaining an order of 10⁵-10⁶ cells·ml⁻¹, being similar to the maximum numbers for the Chromatiaceae species reported for natural H₂S-containing waters from different geographic regions, by keeping the physiological activity at a minimal level. This study aims to confirm the above suggestions by comparing the growth of this bacterium with that of C. buderi strain DSM 176 under light-limited conditions. (Large-celled phototrophic bacterium isolated from Lake Kaiike was referred to as large phototrophic bacterium.)

Preparation of the medium of PFENNIG (1965) and the culture conditions used in this study were already described (MATSUYAMA, 1986, 1987a, b). C. buderi strain DSM 176, which had been designated by TRÜPER and JANNA SCH (1968) for the bacterium isolated from estuarine salt flats on Santa Cruz, Galapagos Islands, was obtained from the German Collection of Microorganisms (Göttingen). Both bacteria could grow well in the NH₄Cl-deficient medium of PFENNIG (1965) by fixing N₂ as a sole nitrogen source, although C. buderi was shown not

Fig. 1 Photomicrographs of the large phototrophic bacterium isolated from Lake Kaiike (A) and Chromatium buderi (B) at logarithmic phases. C. buderi was more motile than the large phototrophic bacterium, and the flagella were visible in the light microscope.
to use N\textsubscript{2} (TRUPER and JANNASCH (1968)). The large phototrophic bacterium was close to *C. buderi* with respect to cell shape and size (Fig. 1). *C. buderi* was very motile; the flagella, about two times the cell length, were visible in the light microscope. Cellular movement of the large phototrophic bacterium was slow, however, and the flagella were hardly observable by microscope. There was a marked difference in the absorption spectrum of living cell suspension between two bacteria; *C. buderi* had two peaks at 800 and 850 nm in the wavelength between 700–950 nm, while the large phototrophic bacterium had a single peak at 830 nm. The *C. buderi* suspension was brown in color, and that of the large phototrophic bacterium was purplish red. The large phototrophic bacterium seems to belong the family Chromatiaceae, because of motility and presence of bacteriochlorophyll *a*, but it did not match any described species (PFENNING and TRUPER, 1983).

Figure 2 (left) shows the growth of *C. buderi* in the batch culture at different light intensities. Continuous light was provided with 100-W incandescent lamps, and light intensity was changed by enclosing each culture bottle with different pore sizes of black nylon net. (The growth of the large phototrophic bacterium under different light intensities was already shown (MATSUYAMA, 1987a)). To the right of the figure are indicated the specific growth rates of *C. buderi* as well as the large phototrophic bacterium estimated from increases in the cell numbers during the logarithmic phases of growth at each light intensity. *C. buderi* grew faster than the large phototrophic bacterium in the wide range of light level examined. But below the light intensity of about 200 lux the growth of *C. buderi* became poor, being comparable to or slower than the large phototrophic bacterium.

As shown in the previous study (MATSUYAMA, 1981), the intensity of light reaching the dissolved O\textsubscript{2}–H\textsubscript{2}S interface of Lake Kaiike was limited, 400–600 lux in a maximum, and it was rapidly attenuated to actually zero just below the interface due to the self shading feature of the dense bacterial popula-
tions (bacterial plate). Most bacterial cells in the plate were suggested to be severely light-limited. To form a dense population the phototrophic bacterium must keep the viability under such a light-limited condition.

Living cell suspensions of the large phototrophic bacterium and C. buderi which had been grown and reached the stationary phases in 1-l glass bottles were distributed into two series of 17-ml test tubes up to the brim. They were tightly stoppered to incubate in the dark at 25°C.

Figure 3 summarizes the changes of the cell numbers, the relative abundances of sulfur-containing cells and the N₂ fixation rates to how long each bacterium keeps its photosynthetic ability; the bacterial N₂ fixation was predominantly controlled by the photosynthesis (Matsuyama, 1986). The N₂ fixation rate was assessed as C₂H₄ formed from 6 ml bacterial suspension injected into a 10-ml syringe, into which 2 ml of 10% C₂H₂ (in argon) and 4 ml of freshly prepared NH₄Cl-deficient Pfennig's medium as H₂S source were added, and the syringe was incubated in the water bath at 25°C under continual light intensity of 2,000 lux for 24 hr. C₂H₄ formed was analyzed with a gaschromatograph (Matsuyama, 1986). Both bacterial suspensions retained the initial cell numbers throughout the experiment. (A continuous culture of large phototrophic bacterium, however, showed a specific growth rate of -0.003·hr⁻¹ in the dark (Matsuyama, 1987b).) But the relative abundance of sulfur-containing cells and the N₂ fixation rate decreased with time. Their decreases were particularly marked in C. buderi. This suggests that the large phototrophic bacterium could survive in the dark for longer than C. buderi; or, more precisely put, the suspension of the large phototrophic bacterium could contain more viable cells than that of C. buderi in the prolonged dark. The large phototrophic bacterium in the dark is suggested to maintain viability in the mode of sulfate reduction as pointed out in some Chromatiaceae species (Hendley, 1955; Trüper and Schlegel, 1964; Trüper and Pfennig, 1966; Gemerdan, 1968a, b), because addition of Na₂MoO₄ 2H₂O (5 mM), an effective inhibitor of sulfate reduction, into the bacterial suspension in the dark retarded the disappearance of sulfur globules from the cells as well as H₂S formation as compared to that without Na₂MoO₄ 2H₂O (Matsuyama, unpubl.).

Figure 4 shows the growth of the large phototrophic bacterium and C. buderi in two series of 70-ml Roux-type flasks with a flat bottom (Corning Culture Flask 25100FK) which were piled up in a water bath at 25°C. The water bath was enclosed with a black sheet, and the flank of each flask was covered with a black nylon tape. Light intensity provided
with a 40-W incandescent lamp hanging above two piles of flasks was adjusted to give 600 lux on the top surface with a 12-hr light-dark cycle. The light intensity decreased exponentially with the number of flasks to bring the light condition close to that at the bacterial plate of Lake Kaiike. The large phototrophic bacterium could increase the cell number in the whole flasks, although the growth rate was generally low, and it decreased downwards. (A similar growth experiment of the large phototrophic bacterium was undertaken previously (MATSUYAMA, 1987b). The growth was limited to the upper four flasks, and it was probably due to a strong effect of self shading of the bacterium, because initial cell number was two orders of magnitude larger than in the present study.) The growth of C. buderi in the upper two flasks, however, was faster than the large phototrophic bacterium. But in the underlying flasks the bacterium could not maintain the initial cell number, although there was no marked difference in light distribution between the two piles of flasks.

From the above data it may be pointed out that C. buderi could not form a dense population under a light-limited condition as at the bacterial plate of Lake Kaiike, probably due to its high requirement for light. The intrinsic low growth rate of the large phototrophic bacterium may be considered as its means of survival under such a light-limited condition, because the lower the growth rate is, the lesser energy the bacterium seems to expend in a dim light or in the dark.

Not only the quantity of light, but also its quality is the important parameter for the formation of dense populations of phototrophic bacteria in natural waters (TRUPER and GENOVESE, 1968; PARKIN and BROCK, 1980). Light quality rapidly changes with depth due to the combined absorbance of water as well as the light-filtering effect of biological communities. Growth experiments of large phototrophic bacterium under the light condition having a spectral composition similar to that at the dissolved O₂-H₂S interface of Lake Kaiike are necessary.

Fig. 4  Growth of the large phototrophic bacterium (left) and C. buderi (right) under different light intensity in the Roux-type flasks, which were piled up. Light intensity was adjusted to give 600 lux on the top surface with a 12-hr light-dark cycle. Light passing the series of flasks was shown as a broken line. Numerals indicates time after inoculation (day). Initial cell numbers were 3.5×10³ and 1.0×10³ cells·ml⁻¹ for the large phototrophic bacterium and C. buderi, respectively.

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