Some Considerations on the Dense Population of a Purple Sulfur Bacterium, *Chromatium* sp., at the Mid-depth of Lake Kaiike

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Abstract

The purpose of the present study is to consider the dense population of a purple sulfur bacterium, *Chromatium* sp., at the mid-depth of Lake Kaiike on Kamikoshiki Island. In summer (1979), the lake was well stratified and at the boundary (4.75 m) between upper O₂ and deeper H₂S layers a dense population (5×10⁶ cells/ml) of *Chromatium* sp. was found. A hyperbolic relation between the bacterial growth and H₂S concentration obtained in the laboratory experiment indicates that the *in situ* growth of *Chromatium* sp. is controlled by the supply of H₂S from the reducing deeper water where it accumulated. The facts that further penetration of light beyond the mid-depth was greatly limited by self-shading of *Chromatium* sp. and that the carbon assimilation at this depth by the bacterium occupied a substantial part of the whole process in the lake suggest that the bacterium also has an important role in the formation and maintenance of the reducing condition of deeper water.

1. Introduction

A stratified lake exhibits many interesting chemical and biological phenomena due to its characteristic physical condition. The bottom part is in great contrast to the upper part and frequently contains an H₂S layer. Sharp gradients of temperature and dissolved O₂ are found between the two layers. Different kinds of microorganisms are noted to be sequentially layered at respective depths to meet environmental and biological requirements without undergoing the disturbance of vertical mixing (GORLENKO and KUZNETSOV, 1972; KUZNETSOV and GORLENKO, 1973; CALDWELL and TIEDJE, 1975a,b; GORLENKO *et al.*, 1978). Hence, a stratified lake offers an ideal field for the study of the relationships between environment and organisms or the biological interactions among different organisms, which are rather difficult to evaluate in well-mixed waters. Due to a limited vertical water motion, specific composition of the phytoplankton that could form stable populations is limited

(FOGG, 1965; HUTCHINSON, 1967). On the other hand, some of the photosynthetic and chemosynthetic bacteria play an important role in organic matter production therein (CZECZUGA, 1968; CULVER and BRUNSKILL, 1969; TAKAHASHI and ICHIMURA, 1970; MATSUYAMA and SAIJO, 1971; COHEN *et al.*, 1977).

The author has made a number of studies on a small stratified lake, Kaiike (MATSUYAMA, 1977, 1978, 1979; MATSUYAMA and SHIROUZU, 1978) on Kamikoshiki Island. The present study deals with evaluating possible mechanism of forming a dense bacterial plate of a purple sulfur bacterium, *Chromatium* sp., at its mid-depth.

2. Methods

The data were obtained from field observations at Lake Kaiike between 31 July and 1 August, 1979, and from laboratory experiments on the growth of *Chromatium* sp., which was isolated from the lake in November, 1978.

Samplings in the lake were made at the

Fig. 1-A

Fig. 1-B

Fig. 1. Vertical distributions of some environmental factors, carbon assimilation rate and *Chromatium* sp. in Lake Kaiike (31 July, 1979). A: vertical distributions of chlorinity, temperature, σ_t , dissolved O_2 and H_2S . B: vertical distributions of relative light intensity, NH_4^+ , PO_4^{3-} , carbon assimilation rates in light (L) and dark (D) bottles, and *Chromatium* sp. Light intensity at 5 m was 0.02 % of just below the surface.

point of a maximum depth, using an acrylic sampler having a 2.5 l capacity. A glass thermometer was used for the measurement of temperature. Chlorinity was determined by the MOHR-KNUDSEN method. Dissolved O_2 was measured by the WINKLER method, but a modified method (INGVORSEN and JØRGENSEN, 1979) was applied at low dissolved O_2 concentration. H_2S was determined by the iodometric titration method of CdS precipitate (AMERICAN PUBLIC HEALTH ASSOCIATION, 1965). Samples for NH_4^+ and PO_4^{3-} determinations were passed through a glass fiber filter and frozen for storage in polyethylene bottles. Both nutrients were determined by the indo-phenol and DENIGÈS meth-

ods, respectively, in the laboratory (OCEANOGRAPHICAL SOCIETY OF JAPAN, 1970). Samples for enumeration of *Chromatium* sp. were kept under cool and dark and cell density was counted with a bright-line haemocytometer within 5 hours after collection. Carbon assimilation rate was measured by the *in situ* method using $^{14}C-HCO_3^-$ (STEEMANN NIELSEN, 1952).

The FULLER's inorganic medium described by BOSE (1963) was employed for isolation and subsequent culture experiments. The composition of the medium and the method of culture were given in the previous study (MATSUYAMA, 1979). The culture was not monospecific, but the biomass of other microorganisms was

judged by microscopic inspection to be less than 1% of the biomass of *Chromatium* sp. The experiments of the effects of H_2S and inorganic nutrients on *Chromatium* sp. growth were conducted with the bacterium in the later exponential growth phase when cell density was nearly of the order of 10^9 cells/ml. Cells were separated from the medium by two successive centrifugations. Then, the bacterium was resuspended into a series of bottles filled with medium from which sulfur, nitrogen or phosphorus was previously depleted. Na_2S , NH_4Cl or NaH_2PO_4 was added to give a series of varied concentrations. Then, the bottles were tightly stoppered and incubated at a constant temperature of $30^\circ C$ and continuously illuminated with incandescent lamps at 2000 lux. After 1-2 days of incubation, the cell density was determined daily with a haemocytometer.

3. Results

1. Field Observations

Fig. 1 shows the vertical distributions of some environmental factors in Lake Kaiike together with the carbon assimilation rate and *Chromatium* sp. in July, 1979.

Chlorinity of surface water was about 7‰, but it increased in the layer from 1 m to 5.5 m depth, where it attained to 18‰. Below this level a slight decrease was found at around 7 m. The bottom water had a chlorinity of 18.42‰. Water temperature was about $31^\circ C$ at the surface and increased notably at 2-2.5 m layer, attaining to a maximum of about $37^\circ C$. Then, temperature decreased rapidly down to 5.5 m. Below this level water had a relatively constant temperature of about $20^\circ C$. σ_t , calculated from chlorinity and temperature, increased consistently in the water from 1 m to 5.5 m, indicating that this layer was stably stratified. Light penetration in Lake Kaiike showed that light easily passed through the upper water,

but below 2.5 m the light transmission deteriorated and was notably poor below 4 m. Dissolved O_2 was saturated in the surface. A marked maximum was observed at the layer from 2 m to 2.5 m. Below this layer it decreased rapidly with depth and ultimately disappeared at 4.75 m. H_2S began to appear at 4 m and increased with depth attaining to a maximum of 19 mg S/l at 5.5 m. Concentrations of HN_4^+ and PO_4^{3-} were low in the upper water, usually less than $1 \mu g$ atom/l. But they accumulated in the deeper water below 4.75 m. Maximum concentrations of NH_4^+ and PO_4^{3-} were 27 and $15 \mu g$ atoms/l, respectively. The accumulation of NH_4^+ in the present observation, however, was slight compared with the previous observations (MATSUYAMA and SHIROUZU, 1978). Carbon assimilation rates by phytoplankton were low, usually less than $50 \text{ mg C m}^{-3} \text{ day}^{-1}$. But a significant assimilation was observed around the O_2 - H_2S boundary at 4.75 m, attaining to $700 \text{ mg C m}^{-3} \text{ day}^{-1}$. *Chromatium* sp. appeared at 4 m and the bacterial density reached a maximum of 5.2×10^6 cells/ml at 4.75 m. Below this level, however, the bacterial density rapidly decreased and the bacterial count was no longer significant at 6 m.

Fig. 2 shows a microscopic photograph of suspended particles at 4.75 m. A substantial part of them was occupied by *Chromatium* sp. cells, although a considerable number of unknown microorganism, having a cell size similar to *Chromatium* sp. but fusiform in shape, was also found.

2. Laboratory Experiments

Fig. 3 shows the growth of *Chromatium* sp. cultured at different temperature between 10 - $38^\circ C$. The bacterium grew fast with increase in temperature up to $32^\circ C$. But above the temperature the bacterial growth rapidly decreased. Relationships between the maximum growth rates

Fig. 2. Microscopic photograph of suspended particles in the bacterial plate of Lake Kaiike. Most of them were occupied by *Chromatium* sp. cells.

(base e) which each curve shows and temperature (reciprocal of absolute temperature) are shown in Fig. 4, in which those for freshwater and marine algae reported by GOLDMAN and CARPENTER (1974) are also given. The relationship for *Chromatium* sp. between 10 and 32°C could be expressed by the equation

$$k = 9.96 \times 10^9 e^{-6278/T}$$

This equation represents a Q_{10} of 2.16. Since the upper limit of growth rates obtained from many cultures of the bacterium under different conditions come to these points, these figures seem to show the maximum one which the bacterium intrinsically has.

Fig. 5 summarizes the growth of *Chromatium* sp. cultured in the media to which different amounts of Na_2S were added as the sole sulfur source. (Most Na_2S will be altered into forms of H_2S and HS^- in terms of the pH of the media.) Many of the culture entered the exponential growth phase immediately after 2 days of

inoculation. The final yields of the bacterium were proportional to the amounts of Na_2S added. Relationships between the amounts of Na_2S and the growth rates of the bacterium at the beginning of the exponential growth phase during 2 and 3 days after inoculation are given in Fig. 6. As seen in the figure, there was a hyperbolic relation and the half-saturation constant (the concentration supporting half the maximum growth rate), which could be obtained from a linear transformation of the MICHAELIS-MENTEN equation, was about 13 mg S/l. GEMERDEN (1974) studied the coexistence of large and small *Chromatium* (*Chr. vinosum* and *weissei*) competing for the same substrate and showed that the MICHAELIS-MENTEN equations exist between the growth rates of both species and Na_2S added, but the half-saturation constants were remarkably smaller than the present one: 0.007 mM for *Chr. vinosum* (maximum growth rate: 0.117 hr^{-1}) and 0.010 mM for *Chr. weissei* (maximum growth rate: 0.040 hr^{-1}).

Figs. 7 and 8 show the growth of *Chromatium* sp. in the media to which different amounts of NH_4^+ and PO_4^{3-} were added as the sole nitrogen or phosphorus sources. As with Na_2S , the final yields of the bacterium were proportional to the amounts of NH_4^+ and PO_4^{3-} added.

4. Discussion

As seen in Fig. 4, *Chromatium* sp. has a lower growth rate than freshwater and marine algae at all temperature examined. If all environmental conditions were optimal, the algae would be the successful competitor in the lake, because of its higher growth rate (GOLDMAN and CARPENTER, 1974). Nevertheless, *Chromatium* sp. has always a substantial role in the organic matter production in the lake and phytoplanktonic production is of secondary importance.

Fig. 3-A

Fig. 3-B

Fig. 3. Effect of temperature on the growth of *Chromatium* sp. A: 10-30°C, B: 30-38°C. Fig. 4. Effect of temperature on the maximum specific growth rate of *Chromatium* sp. Maximum growth rate is to the base e.

As shown in Fig. 6, there exists a hyperbolic relation between the growth rates of *Chromatium* sp. at the initial exponential phase of culture and the amount of Na_2S added; the half-saturation constant was approximated as 13 mg S/l. Hence, *Chromatium* sp. growth would be expected to be closely determined by the amount of H_2S below the half-saturation constant. Actual concentration of H_2S in the layer of the bacterial plate of Lake Kaiike has always been shown to be less than 4 mg S/l, so the *in situ* growth of the bacterium would be obviously controlled by the H_2S supply from the deeper water.

As seen in Fig. 1, inorganic nutrients such as NH_4^+ and PO_4^{3-} were poor in the

upper water above the bacterial plate, but below the plate they accumulated significantly. The deeper nutrient-rich water is covered with the bacterial plate. Since the deeper water of Lake Kaiike is gradually replaced by seawater seeping through the gravel bar, influenced by annual change in water level in the sea, and is lifted and discharged into an adjacent lake in a diluted form (MATSUYAMA, 1978), these nutrients must be rapidly metabolized into bacterial cells at the plate as suggested from Figs. 7 and 8. That is to say, the inorganic nutrients produced in the deeper water seem to be preferentially used at the bacterial plate and only a limited fraction could be used by phytoplankton above it.

Fig. 5. Growth of *Chromatium* sp. incubated in the media to which different amounts of Na_2S were added. The horizontal line indicates the incubation time (days) after inoculation.

As shown in the previous study (MATSUYAMA, 1979), *Chromatium* sp. can grow even under limited light intensity, i.e., as low as about 50 lux, and the bacterium grows fast with increase in light intensity up to about 2000 lux. However, as shown in the same study, the bacterium can thrive only below the algal compensation depth which is usually taken as the depth showing a maximum light intensity of 400-600 lux (ICHIMURA, 1956). Thus, the range of light intensity available for *Chromatium* sp. seems to be limited.

As seen in Fig. 1, incident light was remarkably attenuated in the water below 4 m. *Chromatium* sp. plays a positive role in this reduction of penetrating light, because the bacterium was an important component of suspended particles in this layer (Fig. 2). A significant decrease in light intensity was also noted in the water

immediately above 4 m, and one of the factors involved is probably zooplankton which densely swarmed there to feed on the bacterium (MATSUYAMA and SHIROUZU, 1978).

Therefore, the fact that *Chromatium* sp. is always found as a thin layer horizontally extending over a whole basin covering the surface of the deeper water may be due not only to the fact that the bacterial habitat is restricted below the algal compensation depth, but also to the fact that light penetration beyond this depth is rapidly attenuated by self-shading of *Chromatium* sp. The lower part of the bacterial plate seems to be in virtual darkness.

Since O_2 is not liberated as a waste product in the bacterial photosynthesis as in the photosynthesis of algae, production of organic matter by bacterial photosynthesis below the algal compensation depth and subsequent downward flux of organic matter seem to intensify the dissimilatory processes and bacterial sulfate reduction in the underlying water. [Both processes are probably closely interrelated, as suggested in Lake Nitinat and Lake Suigetsu (RICHARDS *et al.*, 1965; MATSUYAMA and SAJO, 1971).] Furthermore, some species of *Chromatium* have been known to produce H_2S in the dark; they oxidize the reserve food materials coupled with the reduction of previously stored intracellular sulfur to H_2S (TRÜPPER and SCHLEGEL, 1964; GEMERDEN, 1968). So, *Chromatium* sp. at the bacterial plate may be directly or indirectly responsible for the formation of the underlying reducing condition.

At all depths below the algal compensation depth, there is a consumption of dissolved O_2 by respiratory activity of organisms, so an O_2 deficient condition will inevitably appear in the bottom part, if only vertical water motion is limited. Nevertheless, it is doubtful whether, even if the lake is stably stratified, such a

Fig. 6. Relationships between the growth rate of *Chromatium* sp. (k) during 2 and 3 days of incubation shown in Fig. 5 and the amount of Na_2S (S) added to the media. The half-saturation constant (13 mg S/l in this case) is given as the negative S -intercept of the linear regression of S/k vs. S .

Fig. 7. Growth of *Chromatium* sp. in the media to which different amounts of NH_4^+ were added. The horizontal line indicates the incubation time (days) after inoculation.

highly reducing condition appear and persist for a long period without the bacterial plate at its upper margin, because more light will penetrate into the deeper layer, if it is absent. It is, therefore, safe to say that the reducing condition of the deeper part of Lake Kaike is supported by the dense population of *Chromatium* sp. and the growth of the bacterium is also promoted by the underlying reducing condition, preferentially receiving the supply of H_2S and inorganic nutrients. Thus, it may be assumed that even if the stratification of Lake Kaike should be broken for a while, soon after the restora-

tion of stratification, an O_2 deficient condition will inevitably appear near the bottom and it will be changed into a more reducing condition to accumulate H_2S and inorganic nutrients in conjunction with *Chromatium* sp. So, the fact that *Chromatium* sp. has always an important role in the whole organic matter production in the lake in spite of its relatively low growth rate may suggest that such a trophic structure reaches a stable state in the lake in virtue of the stratified condition.

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Fig. 8. Growth of *Chromatium* sp. incubated in the media to which different amounts of PO_4^{3-} were added. The horizontal line indicates the incubation time (days) after inoculation.

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摘 要

この研究は上飯島貝池中層での紅色硫黄細菌, *Chromatium* sp. の密集分布について考察した。夏(1979年), 湖はよく成層し, 上部 O_2 層と下部 H_2S 層との境(4.75 m)には *Chromatium* sp. が濃密に分布(5×10^6 cells/ml)していた。室内実験での *Chromatium* sp. の生長速度と H_2S 濃度との双曲線関係は現場での同細菌の生長が還元層からの H_2S の供給に依存していることを示している。*Chromatium* sp. の自己遮へいによって中層以深への光の透入が抑制されること, 同細菌の炭酸固定作用が湖内の全固定量で重要な部分を占めていることは下部の還元状態

の形成と維持が同細菌によっても支えられていることを示している。

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