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Short Communication

Environmental Conditions Affecting N_2 Fixation by *Chromatium* sp. Bloom in Lake Kaiike

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Two kinds of large-sized cell bacteria, *Chromatium* sp. and *Macromonas* sp., bloom at an upper boundary of the H_2S layer of Lake Kaiike throughout all seasons (bacterial plate). Maximum cell density of *Chromatium* sp. observed in Lake Kaiike was on the order of 10^5-10^6 cells • ml^{-1}, the highest density exhibited by a member of the Chromatiaceae family in natural unpolluted waters[4]. Two requisites for bacterial photosynthesis, light and H_2S, are met only at the upper boundary of the H_2S layer to cause the bloom of *Chromatium* sp. Little information has been presented on environmental factors favoring *Macromonas* bloom.

Chromatium sp. is able to fix N_2 in the presence of light and H_2S, and the N_2 fixation is predominantly controlled by photosynthesis[2]. Thus, the bacterium can grow well by using N_2 as the sole nitrogen source when light is provided at the saturation level (ca. 2,000 lux). However, *in situ* measurement of N_2 fixation by the C_2H_2 reduction method at the upper boundary of the H_2S layer of Lake Kaiike revealed very low N_2 fixation activity[2,3], although the level of photosynthesis was measurable. A question arises what factor is responsible for the observed low rate of N_2 fixation. This study was conducted to find an answer to this question.

Figure 1 shows vertical profiles of the cell density of two bacterial species and environmental variables in Lake Kaiike on 15 December 1992. The methods of lake water sampling, chemical analyses and bacterial counting were the same as described in Matsuyama and Shirouzu[1].

Chromatium sp. and *Macromonas* sp. appeared at 4-5 m depth layers, and reached to the maximum cell density at 6.0-6.5 m depth (6.7×10^5 and 6.2×10^6 cells • ml^{-1}, respectively), and below that layer their cell density decreased sharply. Below 3 m depth, dessolved O_2 decreased with depth and disappeared at 6.5 m depth. H_2S was detected at 5.5 m depth, reaching 1 mM at 10 m depth. Concentrations of NH_4^+ and PO_4^{3-} were very low in the water above 5 m depth (<1 μM). Their concentrations increased gradually with depth, reaching 400 μM (NH_4^+) and 90 μM (PO_4^{3-}) at 10 m depth.

Figure 2 shows the N_2 fixation rate measured by the C_2H_2 reduction method[2,3] of the bacterial plate sample collected from 6 m depth on 15 December 1992. The water samples were filled in a 1-*l* sterile glass bottle and brought to the laboratory under a cool, dark condition. After inverting the samples several times to disperse the bacterial cells uniformly, each 100 m*l* of the samples was introduced into a series of 100-m*l* syringes without exposure to air. A 10 m*l* aliquot of 10% C_2H_2 in

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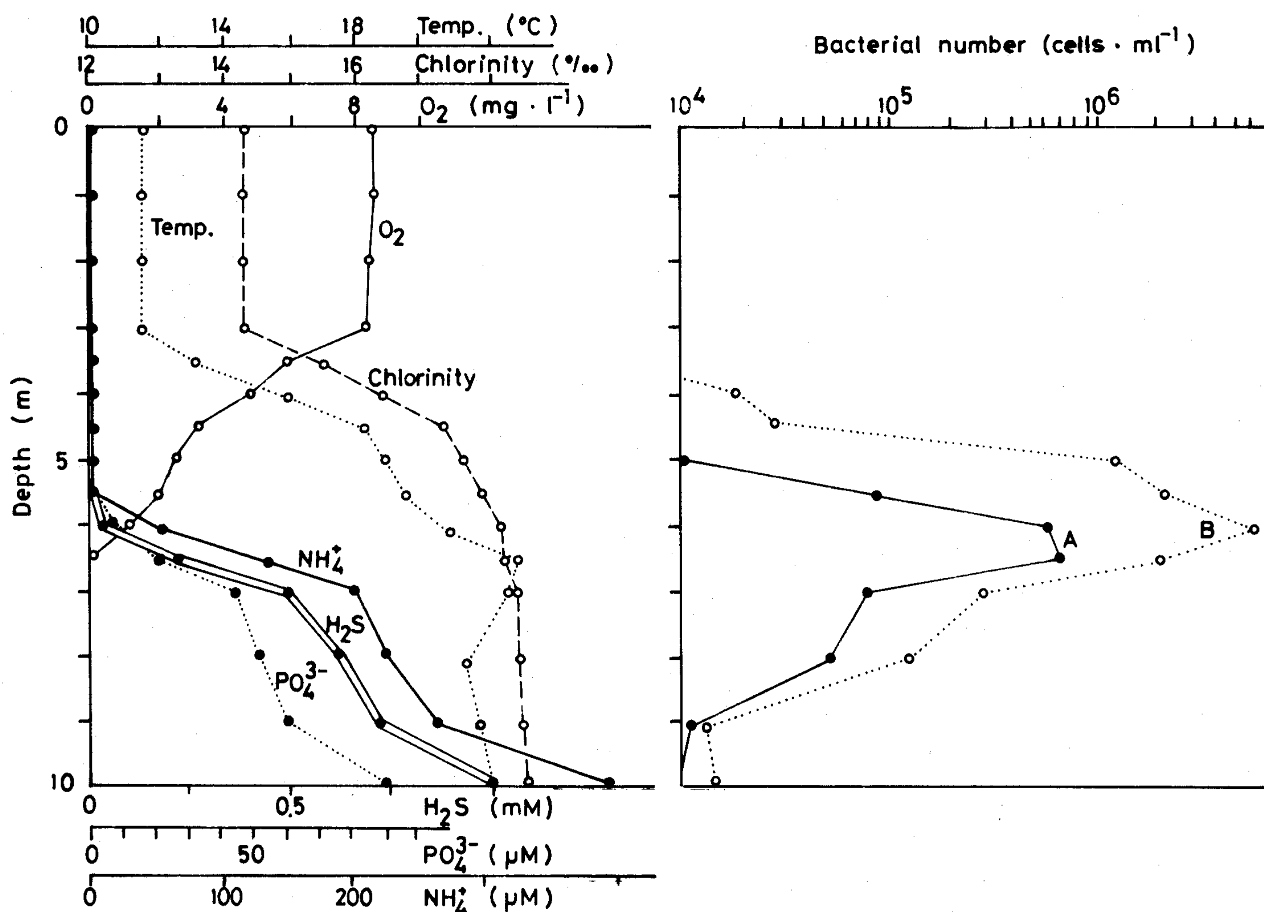

Fig. 1. Vertical profiles of the blooms of *Chromatium* sp. (A) and *Macromonas* sp. (B), and some environmental variables in Lake Kaiike on 15 December 1992.

argon gas was then injected into each syringe, followed by the addition of various amounts of H_2S (as a neutralized Na_2S solution) and NH_4^+ (as NH_4Cl). Within 48 h after collection, they were incubated at 25°C under a continuous light (300 lux) with an incandescent lamp. After 2 days and 5 days incubation, 0.5 m*l* of the gas phase in each syringe was assayed by C_2H_4 measurement using a semiconductor detector-gaschromatograph (Sensortec Inc. Ltd.).

Without H_2S addition, the bacterial plate sample showed limited formation of C_2H_4 (Fig. 2), indicating a limitation of the N_2 fixation by deficiency of H_2S (50 μM) (Fig. 1) and probably a more severe limitation by the presence of NH_4^+ (30 μM) as mentioned later. H_2S addition markedly accelerated N_2 fixation while the simultaneous addition of NH_4^+ depressed it.

Figure 3 shows the change in the C_2H_4 concentration in the pure culture of *Chromatium* sp. Prior to the culture experiment, the bacterium was grown in the inorganic medium of Pfennig[5], which contained 6.4 mM NH_4^+ as a nitrogen source. The bacteria from the exponential growth phase were centrifuged, followed by dispersion in a fresh H_2S- and NH_4^+-free medium and repeated centrifugation. These treatments were done to get rid of both H_2S and NH_4^+ from the cell suspension. The bacterial cells were finally resuspended in H_2S- and NH_4^+-free medium and dispersed into several 100-m*l* syringes. The bacterial suspension was then incubated under a temperature and light condition similar to those employed for the N_2 fixation measurement. During the incubation, a fixed amount of H_2S (0.3 mM) and various amounts of NH_4^+ (0–220 μM) were added daily. The resul-

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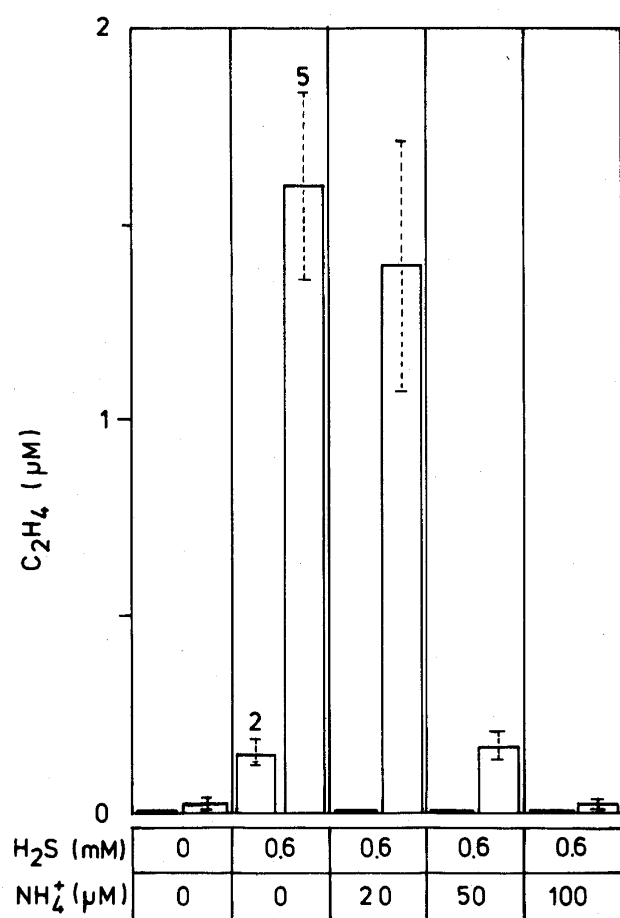

Fig. 2. N_2 fixation rate of the bacterial plate sample of Lake Kaiike added with different amounts of H_2S and NH_4^+. The samples were collected from 6 m depth on 15 December 1992. Each treatment was perfomed in duplicate. Vertical bars represent the range of two replicates. Numbers above the columns indicate incubation time (days).

tant H_2S concentration in the suspension was not markedly different from the *in situ* concentration throughout the experiment.

In the absence of NH_4^+, the N_2 fixation activity of the bacterium was considerably high (Fig. 3). But an addition of NH_4^+ depressed the N_2 fixation, indicating that NH_4^+ was inhibitory for the bacterial N_2 fixation as reported on other N_2 fixing microorganisms[6]. Even a daily addition of 30 μM NH_4^+, one-tenth of that of H_2S, was enough to depress the bacterial N_2 fixation completely.

Increased N_2 fixation of the bacterial plate sample at 5 days incubation in which NH_4^+ was externally added up to 20 μM (Fig. 2) indicates that an addition of 0.6 mM H_2S was sufficient enough to use up environmental NH_4^+ up to 50 μM (initially contained NH_4^+ plus externally added NH_4^+), and remained H_2S was turned to the bacterial use for N_2 fixation. While, limited N_2 fixation of the sample added with 0.6 mM H_2S and 100 μM NH_4^+ was probably due to remaining NH_4^+ after H_2S consumption. The relative abundance of NH_4^+ and H_2S in the bacterial surroundings seems to have a decisive effect on the N_2 fixation. For the occurrence of bacterial N_2 fixation, a significant amount of H_2S must be present after NH_4^+ consumption.

In the water layers below the bacterial plate, H_2S and NH_4^+ occurred at a molar ratio of $\Delta H_2S : \Delta NH_4^+ = 1 : 0.36$ (Fig. 1), which was close to that reported in an anoxic water column of Lake Nitinat[8], suggesting these compounds seemed to be formed by sulfate reduction expressed by the following equation[7]:

$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 53SO_4^{2-} = 106CO_2 + 53S^{2-} + 16NH_3 + 106H_2O + H_3PO_4$, where $(CH_2O)_{106}(NH_3)_{16}H_3PO_4$ is the empirical formula of average organic matter to be decomposed. H_2S production which is required for N_2 fixation of *Chromatium* sp. is accompanied by liberation of NH_4^+ as much as one-third of H_2S on a molar basis. Therefore, as sulfate reduction is the principal process of organic matter decomposition below the bacterial plate, *Chromatium* sp. would never depend on the N_2 fixation.

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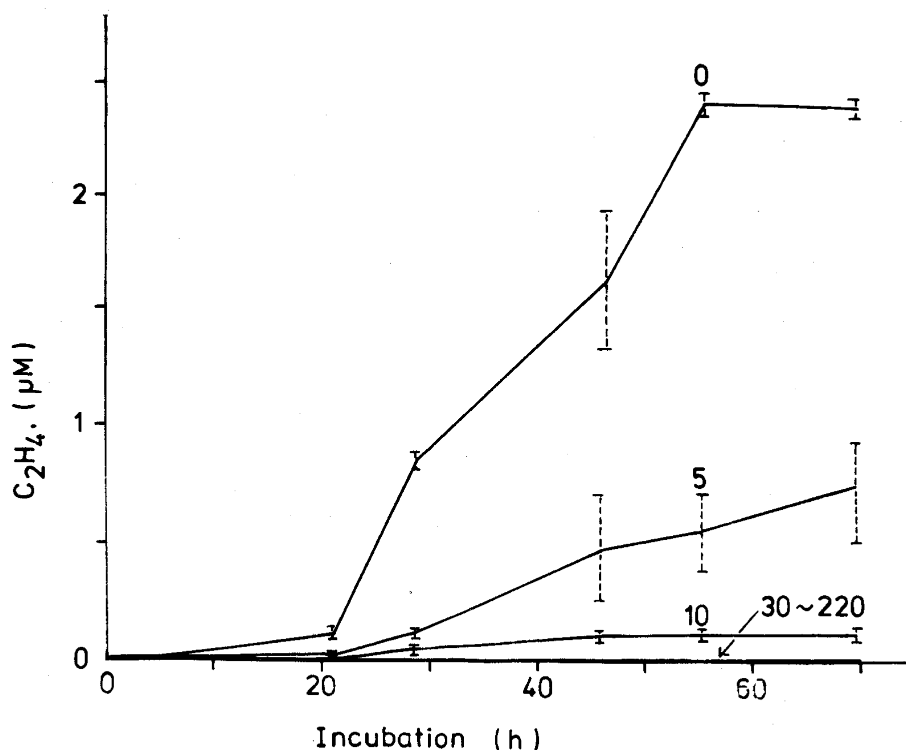

Fig. 3. N_2 fixation rate of the pure culture of *Chromatium* sp. under daily addition of H_2S (0.3 mM) and different amounts of NH_4^+ (0-220 μM). Numbers in the figure indicate added NH_4^+ concentrations. Bacterial density at the start of the culture was 1.8×10^6 cells • ml^{-1}.

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