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<td>Citation</td>
<td>長崎大学水産学部研究報告, v.77, pp.107-110; 1996</td>
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<tr>
<td>Issue Date</td>
<td>1996-03</td>
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<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10069/29775">http://hdl.handle.net/10069/29775</a></td>
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N2 Fixation and Growth of Chromatium sp. with and without NH4+ Addition

Sang-Wook Moon* and Michiro Matsuyama

N2 fixation and growth of Chromatium sp., isolated from Lake Kaiike, were examined with and without NH4+ addition. The bacterial N2 fixation was inhibited by exogenous NH4+. Added NH4+ was rapidly assimilated by the bacterium. The resumption of N2 fixation was found when NH4+ was reduced to a level of 100 μM.

Without the addition of NH4+, the bacterium could grow. However, growth yield added with NH4+ was larger than that without NH4+ addition. Different growth yields between the two nitrogen sources seemed to be strongly related to light and H2S. A low light limited the bacterial growth on N2 resulting in the lowest of all the yields. Intracellular sulfur was shown to be promotable to the bacterial growth on NH4+ rather than that on N2.

Since the bacterial growth on N2 was expected to require more light and H2S than that on NH4+, the bacterial N2 fixation at the site of original habitat in Lake Kaiike was suggested to barely maintain the bacterial number.

Key words: Chromatium sp., N2 fixation, NH4+ addition, Growth yield.

Chromatium sp. is densely populated at an upper boundary of the H2S layer in a stratified lake, Kaiike, throughout all seasons. The dense population of Chromatium sp. at mid-depth of the lake is called the bacterial plate.7 The bacterium is shown to be able to fix N2 at an expense of energy provided by photosynthesis,9 while, for nonphototrophic N2-fixing bacteria exogenous energy supply for reducing N2 is necessary.3,6 N2 fixation of Chromatium sp. and its growth seem to be severely effected by the presence of NH4+.

Inhibitory effect of NH4+ for N2 fixation of the bacterium and the resumption of N2 fixation were examined in terms of molar ratio of H2S and NH4+.7 Because H2S is concurrently consumed by the bacterium during NH4+ assimilation, H2S available to the bacterium after NH4+ consumption is determinative for subsequent N2 fixation.7

Growth by N2 fixation requires an extra energy for reducing N2 to NH4+.8 The bacterial growth on N2 is expected to show different requirement of light and H2S compared to the growth on NH4+ under photolithotrophic growth condition.

The purpose of the present study is to establish an inhibitory effect of NH4+ for the bacterial N2 fixation, and to make clear the difference of the bacterial growth in the media where NH4+ or N2 was added as a sole nitrogen source.

Materials & Methods

Chromatium sp. isolated from the bacterial plate of Lake Kaiike had been cultured at the conditions of 1000 lux, 25°C, pH8.2—8.4 and 4.1 mM of H2S with the medium of Pfennig.8 The NaCl and MgSO4·7H2O in the medium were increased 25 g and 3.5g·l−1, respectively, for marine habitat of the bacterium, and trace element solution SL 7 was replaced with a solution SL 10.9

NH4+-grown bacterial cells in the exponential growth phase were harvested by centrifugation (670 × g, 15 min), and the pellets were resuspended with NH4+-free medium (3 times), and which were used for the measurement of N2 fixation (C2H2 reduction method). Cells washed by H2S- and NH4+-free medium were used for the growths on N2 or NH4+.

For the NH4+-growth, NH4+ stock solution (pH 8.0) was added to the bacterial suspension, while, for the N2-growth a 40 ml of N2 gas was injected as a nitrogen source. A 100-ml syringe was utilized as the culture vessel.

The syringes were placed in a 25°C water bath at 1000 lux. Illumination was provided by 100-W incandescent lamps, perpendicularly positioned over the water bath. A black nylon net was used for obtaining different light levels by rolling around syringes. Culture vessels in water bath were gently agitated and rotated manually at intervals.

In the culture for the NH4+-growth, added NH4+ was utilized and became traceless, and which was possibly found in a stationary growth phase. As a result, dissolved N2 in bacterial suspension was likely to be substituted for NH4+ as a nitrogen source, because the medium was prepared under N2 stream. However, additions of H2S and NH4+ in molar ratio of less than 3 excluded a possibility of the bacterial utilization of N2 for the growth.9

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The bacterial numbers and its relative one containing intracellular sulfur globules were quantified microscopically using a Thoma hemacytometer. In counting work, few drops of 10% formalin solution were used and enough for stopping the bacterial movement and the de novo deposition of intracellular sulfur globules. Measurement of the bacterial N₂ fixation (C₂H₂ reduction method) was performed as previously described. Measurement of NH₄⁺ concentration was done by the Indophenol method.

Known volume of neutralized Na₂S·9H₂O solution was added to the bacterial suspension with a microsyringe. Time-serial changes of H₂S concentration in the bacterial suspension were determined by Cline method.

Results & Discussion

N₂ fixation rate of Chromatium sp. in the different amounts of NH₄⁺ (0–700 μM) is shown in Fig. 1. The rate was decreasing with increasing NH₄⁺ concentration, and completely inhibited by 700 μM. Fig. 2 shows time-serial changes of NH₄⁺ and H₂S concentration in bacterial suspension, and formation of C₂H₄. The bacterium was shown to resume fixing N₂ when NH₄⁺ was reduced to a level of 100 μM.

Fig. 3 shows the bacterial growth with and without NH₄⁺ addition at light levels of 250 and 1000 lux, and concurrent bacterial consumption of H₂S. Without the addition of NH₄⁺, the bacterium could grow, but growth yield added with NH₄⁺ was larger than that without NH₄⁺ addition. Significantly different growth rates were not observed, while apparently different growth yields at the same light intensity were found, showing those differences became larger in low light intensity. Different growth yields seemed to be strongly related to light and H₂S, in connection with kinds of nitrogen sources used for the growth.

At an high light intensity, difference of the bacterial number between both growths began to be large when H₂S in suspension became depleted likely found in a stationary growth phase, shown in Fig. 3. In NH₄⁺-growth at that period, a significant increase in bacterial number was observed, ascribed to the bacterial utilization of intracellular sulfur. However, those increase in N₂-growth was a slight one, which implied intracellular sulfur did not contribute much to a net increase in bacterial number.

At a low light intensity, slow but, continuous growth was observed in NH₄⁺-growth, resulting in a high yield. Even after exhaustion of suspension’s H₂S the growth could be continued for another 3 days at an expense of intracellular sulfur. In N₂-growth, a decrease in growth rate even in the moderate presence of H₂S concentration was found from the 4th day of the incubation (Fig. 3). From that time, only a
Fig. 2. Effect of NH₄⁺ upon N₂ fixation of Chromatium sp. associated with change of H₂S concentration at 1000 lux. Initial concentrations of added H₂S and NH₄⁺ at zero time were 4.6 mM and 313 μM, respectively.

- H₂S concentrations: ●, NH₄⁺ concentrations: ○, C₂H₄ formed.

Vertical bars denote standard deviation of two replicate samples.

Fig. 3. Changes of the bacterial number, relative abundance of sulfur-containing cells and H₂S consumption in culture of Chromatium sp. illuminated with 250 and 1000 lux. Initial H₂S concentrations were in the range of 2.2 to 2.4 mM. In NH₄⁺-growth, NH₄⁺ concentration of 2.3 mM was equally added to the bacterial suspensions.

- Bacterial number grown at N₂ and 250 lux. (●) NH₄⁺, 250 lux. (●) N₂, 1000 lux. (△) NH₄⁺, 1000 lux.

○ Change of H₂S concentration at N₂ and 250 lux. (○) NH₄⁺, 250 lux. (●) N₂, 1000 lux.

△ Change of relative abundance of sulfur-containing cells grown at N₂, 250 lux. (○) NH₄⁺, 250 lux. (○) N₂, 1000 lux.
rapid H₂S consumption with a little increase in bacterial number, which resulted in the lowest of all the growth yields, was occurred.

In a N₂-fixing cyanobacterium, *Aphanizomenon flos-aquae*, of which population was maintained in the surface layer of Wintergreen Lake, it could grow on NH₄⁺ or NO₃⁻ at a low light intensity, but not grow on N₂ at the same light intensity. At a high light intensity the bacterial growth, regardless of nitrogen sources, was dependent on H₂S (Fig. 3), i.e., its growth would respond to an environmentally available H₂S. However, a low light limited the utilization of N₂ for the bacterial growth compared to the NH₄⁺-growth. In another aspect, a low light might limit nitrogenase function or its synthesis.²,³

In conclusion, the bacterium could grow on N₂, however, the N₂-growth required more light and H₂S than the NH₄⁺-growth. In considering in situ light and H₂S conditions in Lake Kailike, the bacterial N₂ fixation is not thought to contribute largely to an increase of the bacterial population in number because of more requirement of light and H₂S for the growth.

In Lake Kailike vertically sharp change in NH₄⁺ concentration within the bacterial plate nicely met with that change in H₂S concentration by their molar ratio of 3.⁷ At an upper part of the bacterial plate H₂S and NH₄⁺ are always in a deficient state.⁶ However, nearly all the bacterium at that place has its intracellular sulfur globules.⁸ Intracellular sulfur in the absence of NH₄⁺ did not largely promote the bacterial growth compared to that in the presence of NH₄⁺ (Fig. 3). At an upper part of the bacterial plate a rapid bacterial growth is not expectable. However, the bacterial N₂ fixation that leads a growth with little increase in bacterial number is likely to be occurred.

**References**

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*: in German.

**: in Japanese with English summary.

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NH₄⁺の添加有無による Chromatium sp. の窒素固定および生長

文　尚郁・松山 通郎

貝池から分離した Chromatium sp. の窒素固定および生長を NH₄⁺ の添加、無添加によって調べた。本菌の窒素固定は添加した NH₄⁺ によって阻害された。添加された NH₄⁺ は本菌によって迅速に同化された。窒素固定の再開は 100μM 以下の濃度で進行した。

NH₄⁺ の添加がなくても本菌は生長したが、一定期間培養した後の生長量は常に NH₄⁺ を添加した方が高かった。それぞれ異なる窒素源による生長量の差は光及び H₂S と強く関連していると考えられた。低照度は本菌の窒素固定による生長を制限し、最も低い生長量をもたらした。細胞内にいなつ粒子は N₂ 生長より NH₄⁺ 生長に対してより促進効果を示した。

本菌の窒素固定による生長は NH₄⁺ による生長に比較し、より多くの光および H₂S を要求することから、貝池の栄養地における本菌の窒素固定はさわじ菌の個体数を維持するものと推察された。