Soft x-ray imaging of *Leptolyngbya tenuis* (*Phormidium tenue*) in Lake Biwa

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Non-stained laboratory-cultured *Leptolyngbya tenuis*, a new name for *Phormidium tenue* [1], inhabiting Lake Biwa, was observed with a soft x-ray microscope at LB-12. In our previous x-ray observation, several granules were clearly seen in the cells [2]. However they have not been identified because a cyanobacteria cell contains several kinds of granules. Immunofluorescence microscopy with an antibody and electron microscopy are effective method to study of phytoplankton. Transmission electron microscopy revealed that cyanobacteria contain inclusion bodies, including cyanophycin, carboxysomes,and polyphosphate bodies. Cyanophycin granules store nitrogen and polyphosphate granules store phosphorus for the bacteria. Carboxysomes are organelles responsible for carbon dioxide fixation. Function of each organelle depends on the spatial arrangement and/or numerical quantity of them. Cyanobacteria control the spatial arrangement of carboxysomes and optimize the metabolic process of carbon fixation [3]. X-ray microscopy has an advantage of imaging cell at natural environment and a role in its identification. Thus soft x-ray observations at various wavelengths were performed to obtain clues to identify the granules in the x-ray image of *Leptolyngbya tenuis*.

Leptolyngbya tenuis was cultured from the water of Lake Biwa and the cells have been observed by soft x-ray microscope (BL-12). Cell suspension without chemical

fixation or staining was dropped onto a polyimide thin film and air-dried. The cells were imaged under atmospheric pressure. Two wavelengths, 2.0 nm and 2.4 nm (below and above the wavelength of K-absorption edge of oxygen, 2.28 nm), were used for the x-ray observation.

Figure 1 shows x-ray microscopic images of *Leptolyngbya tenuis*. Each cell is clearly observed. Granules are also confirmed by 2.0 nm observations clearly. However the granules are not observed by 2.4 nm observations. These results show that oxygen is most important constituent element for the granule. To identify the granule further detailed research with other techniques such as transmission electron microscope is required.

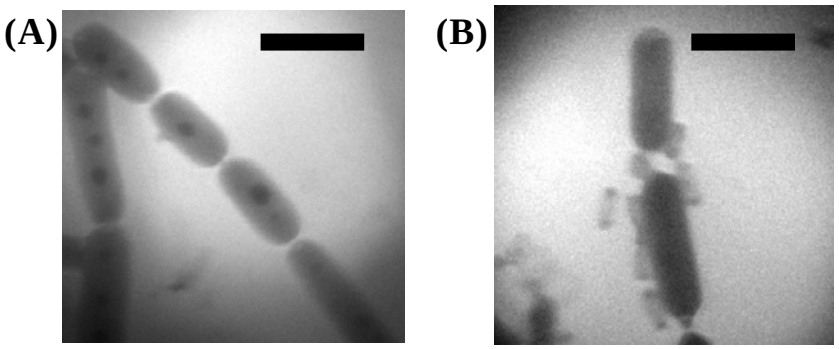

Figure 1 X-ray microscopic images of *Leptolyngbya tenuis*. (A) Observation wavelength is 2.0 nm. (B) Observation wavelength is 2.4 nm. Each exposure time is 5 min. Scale bar is 5 μm.

References

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