X-ray Imaging of Picoplankton in Lake Biwa by Soft X-ray Microscope

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Lake Biwa is the largest lake in Japan and occupies approximately 1/6 of Shiga Prefecture. It provides drinking water for about 15 million people in the Kansai region. The water quality of Lake Biwa has been regularly examined since 1966. Recently, the chemical oxygen demand (COD) index is increasing in spite of a decrease in the values of biochemical oxygen demand (BOD) index [1]. This result suggests that an organic matter which is hard to decompose underwater has been increasing. Photosynthetic picoplankton which is the fraction of the plankton performing photosynthesis composed by cells between 0.2 and 2 μm is considered as an important source of the organic matter. Therefore, X-ray first imaging of a microstructure of the picoplankton inhabiting Lake Biwa was performed.

Laboratory-cultured *Synechococcus* cells, photosynthetic picoplankton, were isolated by centrifugation at 6,200rpm for 3 min at room temperature. The cells were fixed with 1% glutaraldehyde for 30min at room temperature. After fixation, *Synechococcus* cells suspension was placed on a copper mesh with polyvinyl formbar (PVF) membrane and air-dried. When approaching a wet sample observation, a wet sample holder was used [2]. It consists of 2 plates and each plate consists of a thin polyimide film (thickness < 300 μm) and a metal support thin metal plate. *Synechococcus* cells suspension was sandwiched between them, and the X-ray observation was performed at soft X-ray microscope beam line BL12. Observing wavelength is 2.3nm and exposure time is 2min.

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Fig.1 shows air-dried and wet *Synechococcus* cells. In all the micrographs, each cell is clearly distinguishable. Spherical cells and cocoon form cells are observed. The cell of the cocoon form is on cell division. Each cell has a dark sub-micron core. Since a *Synechococcus* cell is covered with gelatin layer, the low contrast region around the core can be interpreted as gelatin layer. From Fig.1 (b), the cell can be estimated at 0.7μm in diameter and gelatin content can be estimate at 1.2μm in diameter. Quantification of gelatin content is required for assessing the water quality in Lake Biwa. Improving a sample preparation and observation technique, gelatin content will be able to be quantified.

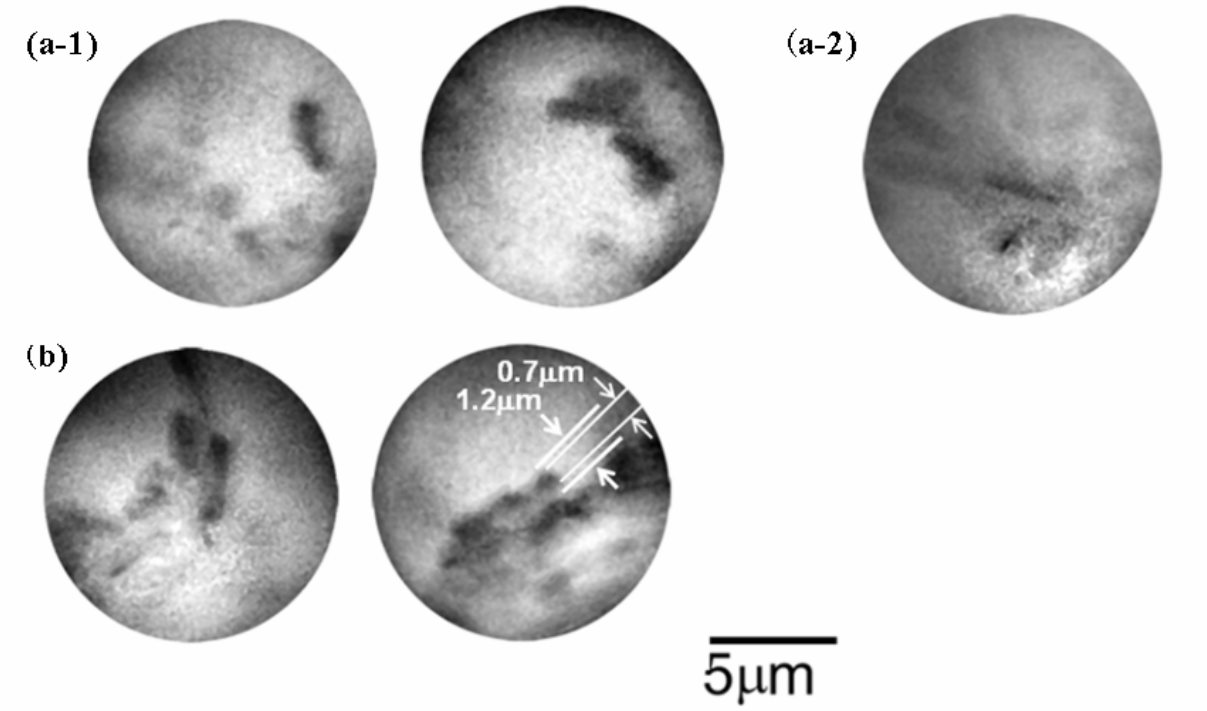

Fig.1 X-ray microscopic images of Laboratory-cultured *Synechococcus* PGS and PP cells. (a-1) Dried and (a-2) wet *Synechococcus* PGS cells, (b) dried *Synechococcus* PP cells.

References

[1] http://www.lberi.jp/root/jp/06db/suisitu/bkjhsuisitu_top.htm#data

[2] K. Takemoto, N. Watanabe, A. Hirai, and H. Kihara: The Object Chamber Staying in Air of the Zone Plate X-ray Microscope, in“X-ray Microscopy and Spectromicroscopy”(*G.Schmahl et al., ed.*), (1998) I129-I134