Soft X-ray Microscopy Observation of Filamentous Cyanobacteria at Various Growth Curve Phase

Kuniko Takemoto[1], Masashi Yoshimura[2], Hidetoshi Namba[2] and Hiroshi Kihara[2, 3]

1) Department of Physics, Kansai Medical University, 2-5-1, Shinmachi, Hirakata, Osaka, 573-1010, Japan

2) SR center, Ritsumeikan University, 1-1-1, Noji-Higashi, Kusatsu 525-8577, Japan

3) Himeji Hinomoto College, 890 Koro, Kodera-cho, Himeji, Hyoto 679-2151, Japan

1. Introduction

Lake Biwa provides tap water to over 14 million residents in the Keihan region. In 1969, a problem of musty smell in tap water has occurred due to sudden propagation of a certain green filamentous cyanobacterium. The filamentous cyanobacterium was reported as *Phormidium tenue* (Menegh.) Gomont and 2-methylisoborneol (2-MIB) was identified as a causative substance [1]. However, results of our soft X-ray microscopy (XM) observation suggested the necessity of reidentifying of it. Recently, it has been reidentified as *Pseudanabaena foetida nom. nud*. based on TEM observation and genetic analysis [2]. To provide an effective maintenance management for water quality of reservoir, understanding of environmental factors and growth curve phase which influence 2-MIB production are needed. However, there is little information about them. To obtain a microstructure at various growth curve phase, *P. foetida* was observed using a soft XM with much higher resolution than conventional light microscope.

2. Experimental

P. foetida which was isolated from Lake Biwa was grown in the conventional culture condition [3]. The cultures were illuminated with fluorescent lamps, which provided 100 μmol photons m^{-2} s^{-1} of photosynthetically active radiation. The XM observation was performed using the BL-12. The cell suspension dropped onto a polyimide thin film (thickness < 350 nm) [4] or silicon nitride (SiN) thin film (thickness: 100 nm) (SIRN-5.0-200-1.0-100, Silson Ltd.). After air-dried, the samples were observed by XM at a wavelength of 2.0 nm and 2.33 nm at room temperature. TEM observation was also performed using JEOL JEM 1400 electron

microscope. Agar embedded cells were cut into small cubes and postfixed. After dehydrating, the same method as in the previous report was carried out [4].

3. Results and Discussion

Figures 1 show X-ray micrographs of air-dried *P. foetida*. Two cells are shown. Focusing on the left side cell, two characteristic structures are observed. One is a granular structure (black arrowheads) and the other is an irregularly shaped structure (black arrows). The irregularly shaped structures are also observed in the right side cell. The granular structures are only observed at 2.0 nm (the shorter wavelength region close to the oxygen K-edge). In previous paper, our investigation indicated that the granule was identified as a microstructure containing oxygen element, such as polyphosphate granule [4]. The irregularly shaped structures are observed at both 2.0 nm and 2.33 nm (the longer wavelength region close to the oxygen K-edge). The irregularly shaped structures have been observed during cell division period, while the granular structures have not been observed during the period. Although prokaryotic cells lack a nucleus, they have a nucleoid. The nucleoid is an irregularly shaped region within the prokaryotic cell (Fig. 2). In addition, carbon is a main component of the nucleoid and the concentration of nucleoid depends on the various cell cycle phases. The result suggests that the irregularly shaped structure is identified as a nucleoid.

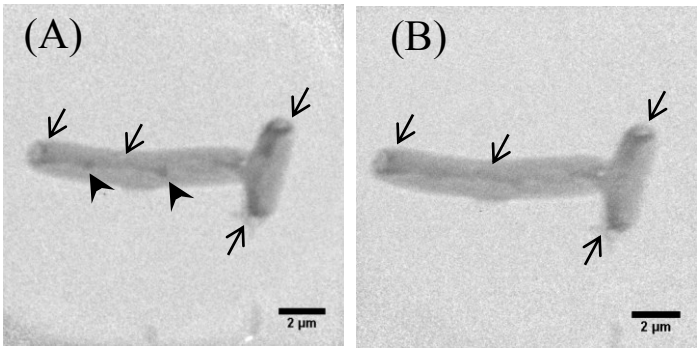

Fig. 1 XM images of *P. foetida*. observed at 2.0 nm (A) and 2.33 nm (B). Black arrowheads show granular structures and black arrows show irregularly shaped structures.

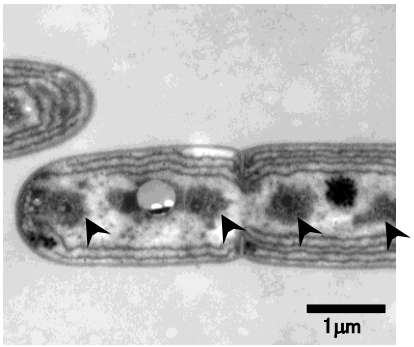

Fig. 2 TEM image of *P. foetida* on cell division. Black arrowheads show nucleoid.

References

[1] M. Yagi, et al., *Water Sci. Technol.*, **15**, 311-321 (1983).

[2] Y. Niiyama, et al., *Fottea*, **16**, 1-11 (2016).

[3] M.M. Watanabe and T. Ichimura, *Bull. Jpn. Soc. Phycol*. **25**, 371-377 (1977).

[4] K. Takemoto et al., *AIP Conf. Proc*. **1696**, 020024 (2016).