

Soft X-ray Imaging of Microstructure of *Phormidium tenue* under Different Culture Conditions

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In the 1970s, a problem of musty smell in drinking water has occurred due to sudden propagation of certain plant plankton in Lake Biwa. *Phormidium tenue* (*Pseudanabaena* sp. [1]) was identified as a causative alga. In the *P. tenue*, it is known that there is a strain not producing musty smell. Such *P. tenue* could not be distinguished under standard light microscopic examination. Musty odor producing and non-producing *P. tenue* were observed with a soft x-ray microscope (XM) with much higher resolution than light microscope. [2-4]. In order to identify intracellular structures recognized by the soft XM observation, a transmission electron microscopy and a low temperature / low vacuum scanning electron microscope (low temperature / low vacuum SEM) were also applied [4]. While musty smell producing strain (green strain) was composed of thick cells without mucilaginous sheath, musty smell non-producing strain (brown strain) was composed of slender cells with mucilaginous sheath. It is possible to distinguish musty odor producing and non-producing strain based on the morphological feature using XM.

In a field study, it is known that the shape, size, and microstructure of a cyanobacteria cell depend on their habitat condition, such as water flow, water temperature, water depth and clarity. The following experiments were conducted in order to investigate the influence by the flow of the water to cell growth. *P. tenue* cells producing musty smell were isolated from Lake Biwa and cultivated were grown in static CT medium or in CT medium on a rotary shaker. A proliferative condition was confirmed by musty smell and color. Cell

suspension without chemical fixation and staining was dropped onto a polyimide thin film (thickness < 300 nm) and air-dried. The cells were observed at soft XM beamline (BL-12) under atmospheric pressure. The x-ray images were taken with a wavelength of 2.0 nm (below the wavelength oxygen K-edge threshold, 2.28 nm).

Figure 1 shows x-ray micrograph of *P. tenue*. A single-trichome without sheath is clearly observed. A granule which is a characteristic structure of *P. tenue* in XM observation is also clearly recognized [2-4]. The large difference was not recognized in the size distribution of the granule between the two. The cells grown in the static medium (Fig. 1 A) look thicker than that grown in the medium on the rotary shaker (Fig. 1 B). The average cell widths of Figs. 1 A and B were 1.69 μm and 1.35 μm , respectively. The length of the filamentous cyanobacteria grown in the static medium was quite longer than that grown in the medium on the rotary shaker although figures were not shown in this paper. In a field work, it will have the influence in growth of cyanobacteria because water flow is always produced by a river and a landform of lake. Stagnant water causes multiplication of *P. tenue*, contributing to deteriorate water quality.

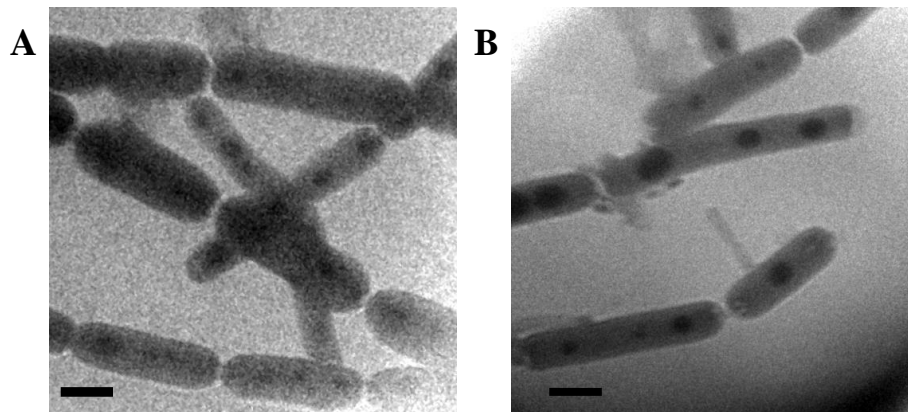


Figure 1 X-ray micrographs of *P. tenue* grown in static CT medium (A), and grown in CT medium on a rotary shaker (B). Exposure times of A and B are 180 s and 120 s, respectively. Scale bar: 2 μm .

References

- (1) Y. Niyama, *Japanese Journal of Limnology*, **2012**, 73, 187-196 (in Japanese).
- (2) K. Takemoto, S. Ichise, T. Ohigashi, H. Namba and H. Kihara, *Memoirs of SR Center Ritsumeikan Univ.*, **2010**, 12, 169-170.
- (3) K. Takemoto, S. Ichise, T. Ohigashi, H. Namba and H. Kihara, *Memoirs of SR Center Ritsumeikan Univ.*, **2011**, 13, 205-206.
- (4) K. Takemoto, A. Yamamoto, G. Mizuta, S. Ichise, M. Yoshimuar, H. Namba and H. Kihara, *Journal of Water and Waste.*, **2012**, 48, 157-163 (in Japanese).