

## Microstructure and 2-MIB production of *Pseudanabaena foetida* sp. (*Phormidium tenue*)

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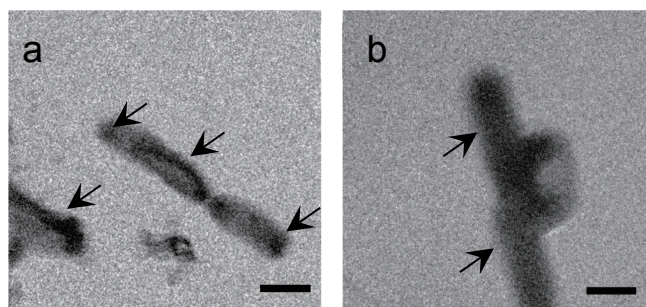
Last summer, musty smell of tap water supplied from Lake Biwa caused a great trouble in the southeastern part of Shiga prefecture. Since 1969, the problems have frequently occurred in Keihanshin area due to sudden propagation of a certain green filamentous cyanobacterium that produces 2-methylisoborneol (2-MIB). *Pseudanabaena foetida* nom. nud. (*Phormidium tenue* (Menegh.) Gomont) is one of the causal cyanobacterium. The aim of this study is to elucidate the microstructure related to the 2-MIB productivity of *P. foetida*.

*P. foetida* which was isolated from Lake Biwa was grown in the conventional culture condition [1]. The cultures were illuminated with fluorescent lamps, which provided  $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  of photosynthetically active radiation. The XM observation was performed using the BL-12. The cell suspension dropped onto a silicon nitride thin film (thickness: 100 nm). After air-dried, the samples were observed by XM at room temperature.

Figure 1 shows typical X-ray micrographs of air-dried *P. foetida* cultured for 2 weeks (a) and 12 weeks (b). Images were taken at 532 eV (2.33 nm). In Fig.1 (a), distinct granular structures referred to as polyphosphate granules are not seen. However, several intracellular structures are observed, black arrows. It is well known that oxygen K-edge XANES spectra show a peak attributed to the carboxyl group ( $\text{O-C=O}^*$ ) at 530-535 eV. It is suggested that the intracellular structure includes the carboxyl group,  $-\text{COOH}$ , in its chemical structure and its activity is high. As shown in Fig.1 (b), a cell loses its distinct outline. The cells show a uniform contrast, black arrows. It is suggested that the cell does not contain any prokaryotic organelle-like structure and its activity is low.

The 2-MIB production per cell increased depending on culture period. The ratio of dissolved 2-MIB (including extracellular and dissolved intracellular 2-MIB) to the 2-MIB production was almost the same except for 12 weeks culture. After 12 weeks culture, the ratio increased

rapidly. Long-term cultivation, 12 weeks, can cause growth arrest, resulting in damage to the cell. The intracellular 2-MIB can easily leak through the broken cell wall. However, when it is a normal cell, the same amount of 2-MIB is excreted to the exterior of cell during the same period. These results support our XM observation results.



**Fig.1** XM images of air-dried *P. foetida* cells cultured for 2 weeks (a) and 12 weeks (b). Images were taken at 532 eV, 2.33 nm. Exposure time was 120 s. Scale bar is 2  $\mu$ m.

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#### **References**

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