Soft X-ray Microimaging of Musty-Odor Producing Filamentous Cyanobacterium in Exponential Phase

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Lake Biwa provides potable water to over 14 million residents in the Kinki region. Although it is therefore important to maintain high water quality, a problem of musty smell in drinking water has occurred due to sudden propagation of a certain green filamentous cyanobacterium since 1969. The filamentous cyanobacterium was identified as *Phormidium tenue* and 2-methylisoborneol (MIB) was identified as a causative substance [1]. Recently, however, it has been reidentified as *Pseudanabaena foetida* nom. *nud.* based on the TEM observation and genetic analysis [2, 3]. Although a large number of studies have been made on musty-odor substance, little is known about *P. foetida*. *P. foetida* is a small filamentous cyanobacterium without a sheath, and the sticky trichome (hormogonia) [2, 5]. To obtain detailed microstructural information, *P. foetida* was observed using a soft X-ray microscope (XM) with much higher resolution than light microscope [4, 5]. In previous experiments, long sticky trichomes without sheath were observed in cells in the stationary phase (Fig. 1). Heterocysts for nitrogen fixation and akinetes (spores) for survival were not observed. A few granules were observed in each cell.

The following experiments were conducted to investigate microstructure of *P. foetida* in a logarithmic growth phase. Laboratory-cultured *P. foetida* cells in the stationary growth phase were used as seeds. The seeds were cultivated in the conventional culture condition [6]. To obtain cells in the logarithmic growth phase, cell suspension was extracted after 1 and 2 weeks cultivation. Each cell suspension without chemical fixation or 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. X-ray images were taken with a wavelength of 2.0 nm (below the wavelength oxygen K-edge threshold, 2.28 nm) and 2.33 nm (above the wavelength oxygen K-edge threshold).

Cell number was estimated by a Multisizer III (Beckman Coulter). The number of 1 week and 2 week cultured cells were approximately 17 and 27 times larger than that of seeds, respectively. Figure 2 shows X-ray micrographs of *P. foetida* of 2 weeks cultivation. Trichomes and granules are clearly observed [4, 5]. However, long trichomes are not confirmed. The cell numbers after 1 and 2 weeks cultivation were obtained to be 1.4 and 1.3 per unit of trichome, respectively. The cell number of the seed was obtained to be 2.2 per unit of trichome. These results suggest that the disconnection precedes the cell division in the exponential growth phase. In addition, besides granules, a characteristic microstructure was observed. The microstructure was observed not only at 2.0 nm, but also at 2.33nm. Currently, the identification of the microstructure has been carrying on.

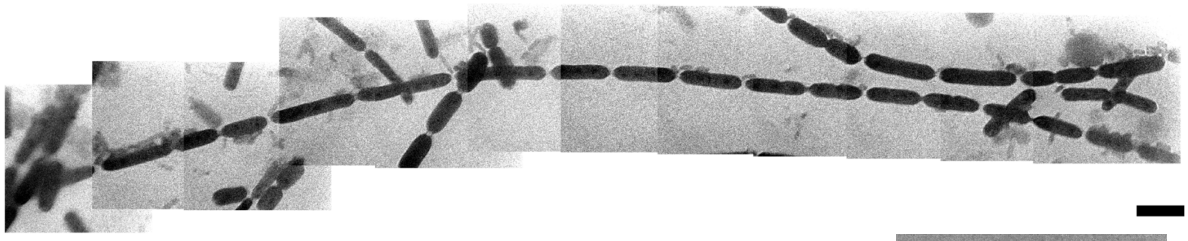

Figure 1 X-ray micrograph of *P. foetida* in the stationary phase

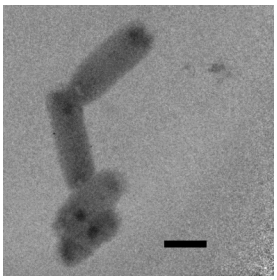

Figure 2 X-ray micrograph of *P. foetida* after 2 weeks cultivation. Scale bar: 2 μm.

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