Response of *Shewanella oneidensis* Strain MR-1 to Fe(II)-Induced Inhibition of Anaerobic Respiration

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Dissimilatory iron metal-reducing bacteria control the biogeochemistry of anaerobic, nonsulfiogenic environments principally by reducing Fe(III) to Fe(II). The products of this respiratory metabolism are generally recognized as significant contributors to redox reactions that can mediate the degradation or immobilization of certain classes of contaminants, including halogenated organic compounds, nitrate, heavy metals, and radionuclides. However, the products of iron respiration can also impact cellular metabolic activity through mechanisms that are analogous to suffocation in higher organisms.

We previously reported that Fe(II) accumulates on the surfaces of iron-reducing bacteria and that this accumulation inhibited both anaerobic respiration and cell growth. Electron microscopic images revealed that Fe(II)-rich mineral precipitates were removed from cell surfaces by an unknown mechanism. Removal of the precipitates coincided with restoration of cellular respiration and growth. We have extended our investigation to include controlled cultivation methods (chemostats) that allow continuous monitoring and independent control of culture parameters. *Shewanella* was continuously cultured using a defined medium with lactate as the electron donor and fumarate as the electron acceptor. Ammonium was provided as the sole nitrogen source at a growth-limiting concentration. To investigate the response of cells to Fe(II)-induced inhibition, an anaerobic solution of $FeCl_2$ was added to the steady-state cultures to a final concentration of 1 mM, which was sufficient to saturate cell surfaces. Samples for transmission electron microscopy (TEM), mRNA microarray analysis, and Mossbauer spectroscopy were taken at regular time intervals after the addition of Fe(II). TEM revealed the accumulation of iron precipitates evenly distributed over cell surfaces. In the presence of excess soluble electron acceptors (fumarate), the precipitates began to clear within 1 hr after their formation. Surface blebs were also formed over the entirety of the cell surface, as previously documented. However, removal of surface precipitates was not mediated by these blebs, as suggested by our previous work. Instead, the iron precipitates appeared to be internalized by the cells and partially transformed to a less electron-dense phase. Within 2 hr after Fe(II) addition, cells were completely free of cell surface precipitates. The extracellular medium was dominated by protein-rich structures. The protein complement of these structures is being examined by MS-based proteome analysis. Cellular response to Fe(II)-induced respiratory stress at the transcriptome level is underway using mRNA microarray technologies.

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