Display and Retraction of Outer Membrane Cytochromes by *Shewanella oneidensis* in Response to Electron Acceptor Availability

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Morphological and compositional analyses of the surface of Shewanella oneidensis strain MR-1 were conducted and compared under conditions of electron acceptor limitation and electron acceptor repletion. Atomic force microscopy (AFM) reveals that cells display nanoscale domains that protrude through the outer membrane and bristle the bacterial surface when availability of electron acceptor is limited. These protrusions disappear within seconds from cell surfaces after electron acceptor limitation is relieved by adding oxygen, nitrate, fumarate, or chelated forms of ferric iron. Mutants of MR-1 that do not produce outer membrane secretin-forming protein (GspD) associated with the type II secreton (T2S) system do not produce protrusions under either electron acceptor-limited or excess conditions. Surface-enhanced Raman spectroscopy (SERS), a technique that selectively amplifies Raman vibrational signals from molecules located within a few angstroms of thin films composed of elemental silver or gold, revealed that heme-containing proteins (cytochromes) are selectively exposed on the surface of wild type MR-1 cells in response to acceptor limitation. Surface-exposed heme was not detected under either electron-acceptor-limited or excess conditions for a mutant lacking the GspD secretin. Correlation of data from AFM and SERS supports our hypothesis that cytochromes, possibly those previously implicated in solid phase iron and manganese oxide reduction, are substrates of the T2S and may form a functional protein complex that extends and retracts through the outer membrane in direct response to electron acceptor availability. A physiology-based model describing the control and distribution of redox-reactive heme proteins on the MR-1 cell surface is presented.