Identification of a c-type cytochrome involved in Mn(IV) reduction in Anaeromyxobacter dehalogenans strain 2CP-C

Frank Loeffler (frank.loeffler@utk.edu) - University of Tennessee & Oak Ridge National Laboratory (PI), Susan Pfiffner, University of Tennessee; Robert Hettich, Oak Ridge National Laboratory (Co-PIs).

Anaeromyxobacter dehalogenans strains are metabolically versatile Deltaproteobacteria that conserve energy from the reduction of a variety of electron acceptors, including insoluble manganese dioxide (MnO2) and ferric oxides/oxyhydroxides (FeOOH). The goal of this research effort was to identify c-type cytochromes involved in electron transfer to the insoluble electron acceptor MnO2.

The characterization of deletion mutants has revealed a number of c-type cytochromes involved in electron transfer to solid metal oxides in Shewanella spp. and Geobacter spp; however, knockout mutants often do not display distinct phenotypes indicating the limitations of this approach for making functional assignments to specific c-type cytochromes. A genetic system for Anaeromyxobacter is not available and functional assignment of c-type cytochromes is lacking. To identify A. dehalogenans c-type cytochromes involved in electron transfer to solid metal oxides, protein expression profiles of strain 2CP-C cells grown with acetate as electron donor and MnO2, ferric citrate, ferric oxyhydroxide, nitrate or fumarate as electron acceptors were compared. Whole cell lysates were subjected to trypsin proteolysis and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Distinct c-type cytochrome expression patterns were observed in cells grown with the different electron acceptors. A. dehalogenans strain 2CP-C cells grown with insoluble Mn(IV) expressed 25 c-type cytochromes out of the 69 c-type cytochromes encoded on the strain 2CP-C genome. An uncharacterized c-type cytochrome (Adeh_1278) was uniquely expressed in strain 2CP-C cells grown with MnO2. PCR and reverse transcriptase-qPCR demonstrated that the Adeh_1278 gene was transcribed in cells grown with MnO2 but not with any of the other tested terminal electron acceptors. Apparently, the transcription of the Adeh_1278 gene and Adeh_1278 expression correlated with Mn(IV) reduction activity in A. dehalogenans strain 2CP-C. Adeh_1278 has three heme binding motifs and is predicted to occur in the periplasm. The identification of Adeh_1278 as a protein uniquely expressed during growth with MnO2 suggests its utility as a biomarker for MnO2 reduction and demonstrates the value of the LC-MS approach for making functional assignments to c-type cytochromes. The distinctive expression of c-type cytochromes in response to growth with different terminal electron acceptors offers opportunities for functional (i.e., activity) in situ monitoring using environmental metaproteomics or transcript-targeted approaches.