ABSTRACT: The project goal is to utilize innovative proteomics tools to generate new information regarding microbial activity contributing to metal and U(VI) reduction, and thus enable science-based decision making for long-term site management. Current objectives are to: (1) examine c-type cytochromes in *Anaeromyxobacter dehalogenans* strain 2CP-C, *Shewanella oneidensis* strain MR-1, and *Geobacter daltonii* strain FRC-32 under different growth conditions and develop a sequence library comprised of c-type cytochromes implicated in metal reduction and other redox processes; (2) examine the proteomes of these strains and identify proteins that correlate with metal reduction; and (3) apply proteomic analyses to ORIFRC field samples to demonstrate the utility of this approach for site assessment, efficient implementation of bioremediation and long-term monitoring. We used a tiered approach that combines database mining, controlled laboratory studies, and PCR approaches to support the proteomics characterizations.

c-Type cytochromes are heme-containing proteins involved in electron transfer to oxidized metal species. The genomes of *A. dehalogenans* strain 2CP-C, *S. oneidensis* strain MR-1, and *G. daltonii* strain FRC-32 encode 69, 40, and 72 c-type cytochromes, respectively. Distinct c-type cytochrome expression patterns were observed in cells grown with the different electron acceptors. Our proteome measurements revealed that the number of c-type cytochromes identified in Fe(III) and Mn(IV) grown cells were 19 and 20 (out of 40) for strain MR-1; and 27 and 25 (out of 69) for strain 2CP-C. Proteomic characterization of c-type cytochrome expression revealed substrate-dependent responses suggesting that c-type cytochrome profiling provides information about cellular metabolic activity.

To extend the proteome approach to field samples, proteomic analysis was performed on groundwater filters from Area 2 of the ORIFRC which were collected 4 days after the emulsified vegetable oil (EVO) amendment. The altered groundwater community in EVO amended well was dominated by members of the *Betaproteobacteria* (i.e., *Dechloromonas*, *Ralstonia*, *Rhodoferax*, *Polaromonas*, *Delftia*, *Chromobacterium*) and *Firmicutes*. When metaproteomic workflows were applied to early biostimulation (4 days after EVO injection), distinct differences in protein expression were observed between groundwater collected from wells up-gradient and down-gradient of the EVO injection gallery. In the biostimulated sample the prominent proteins were categorized as being proteins involved in ammonium assimilation, nitrous oxide reduction, EVO degradation, and polyhydroxybutyrate formation. c-Type cytochromes and citrate synthase, which is a biomarker for hexavalent uranium reduction activity, were detected in low abundances suggesting that metal reduction has not commenced 4 days following EVO injection. Thus environmental metaproteomics provided valuable information and complemented nucleic acid-based approaches for identifying microbial community responses to biostimulation and elucidating active metabolic pathways.