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Recipient Name: Cornell University

Project Title: Improved Understanding of Microbial Iron and Sulfate Reduction Through a Combination of Bottom-up and Top-down Functional Proteomics Assays

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Executive Summary

This report summarizes the successful outcomes of our collective studies resulting from our DOE-funded project. Our research led to a more complete understanding of iron and sulfate reduction carried out by phylogenetically diverse iron and sulfate reducing bacteria (IRB and SRB), and developed novel tools and workflows for discovery and monitoring of microbial iron and sulfate reduction. These outcomes resulted from the combination of three complementary phases of research, beginning with biological process characterization of proteins that lead to more in-depth functional characterization using both top-down and bottom-up proteomics characterization combined with heterologous expression of putative reductases in *E.coli*. In this final report we highlight our success including:

- Identification of core genes specific to IRB and SRB in the gamma-proteobacteria, delta proteobacteria and Firmicutes and proteome expression profiles on iron and alternate electron acceptors for *Anaeromyxobacter dehalogenans, Geobacter sulfurreducens, G. bemidjiensis, Shewanella oneidensis,* and the Firmicute, *Desulfotomaculum reducens.*
- Development of a novel workflow for nondenaturing separation and screening of iron reductase enzymes.
- Development of new cloning techniques for heterologous expression using host-ligation systems.
- Heterologous expression and functional characterization of a novel NADH:flavin oxidoreductase from *Desulfotomaculum reducens* capable of IR (as well as Uranium and Chromium reduction). These proteins were the first discovered in a Gram (+) microorganism.
- Publishing the first global proteome comparison of a Gram (+) bacterium, *Desulfotomaculum reducens*, capable of both IR and SR.
- Discovery of two c-type cytochrome that were significantly upregulated under growth on iron in the poorly characterized Delta-proteobacteria, *Anaeromyxobacter dehalogenans*.
- Development of quantitative biomarker assays for all known uranium reductases that can be used on lab cultures as well as to assess different populations of iron/uranium reducers and contaminated field sites.
- Discovery that iron reduction and electron donor processing was faster in *Geobacter sulfurreducens-Desulfotomaculum reducens* co-cultures then in either species grown in pure culture. We also show that both organisms contribute to iron reduction.

We have had considerable success as a multi-disciplinary collaboration having: 1) published 5 papers in high-tiered journals (two more manuscripts are in preparation), 2) supported two graduate theses (one PhD and one MS student), 3) released a comparative genomics software publically, and 4) trained two undergraduate students.

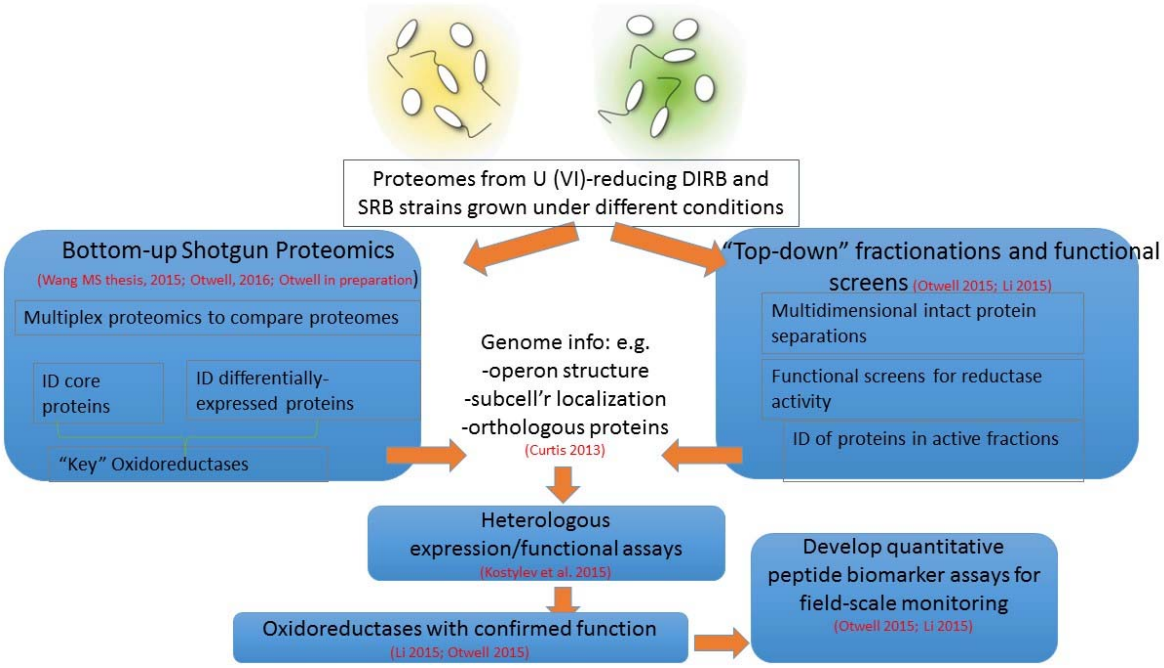

Figure 1. Overview of work tasks in the project and publications resulting from the research.

Goals and Objectives: Relevant Accomplishments

Our overall goal was to improve the understanding of microbial iron and sulfate reduction by evaluating a diverse iron and sulfate reducing organisms utilizing a multi-omics approach combining "top-down" and "bottom-up" omics methodologies. We initiated one of the first combined comparative genomics, shotgun proteomics, RTqPCR, and heterologous expression studies in pursuit of our project objectives. Within the first year of this project, we created a new bioinformatics tool for ortholog identification ("SPOCS"). SPOCS is described in our publication, Curtis et al., 2013. Using this tool we were able to identify conserved orthologous groups across diverse iron and sulfate reducing microorganisms from Firmicutes, gamma-proteobacteria and delta-proteobacteria. For six iron and sulfate reducers we also performed shotgun proteomics ("bottom-up" proteomics including accurate mass and time (AMT) tag and iTRAQ approaches). Cultures include Gram (-) and Gram (+) microbes. Gram (-) were: *Geobacter sulfureducens* (grown on iron citrate and fumarate), *Geobacter bemidjiensis* (grown on iron citrate and fumarate), *Shewanella oneidiensis* (grown on iron citrate and fumarate) and *Anaeromyxobacter dehalogenans* (grown on iron citrate and fumarate). Although all cultures grew on insoluble iron, the iron precipitates interfered with protein extraction and analysis; which remains a major challenge for researchers in disparate study systems. Among the Gram (-) organisms studied, *Anaeromyxobacter dehalogenans* remains the most poorly characterized. Yet, it is arguably the most versatile organisms we studied. In this work we have used comparative proteomics to hypothesize which two of the dozens of predicted c-type cytochromes within *Anaeromyxobacter dehalogenans* may be directly involved in soluble iron reduction. Unfortunately, heterologous expression of these *Anaeromyxobacter dehalogenans* c-type cytochromes led to poor protein production and/or formation of inclusion bodies, even when we co-expressed several genes known to be important for assembly of cytochrome holoenzymes. We confirmed the proteomics trends at the RNA level by designing specific primer sets for

hypothesized iron reductases in *Anaeromyxobacter dehalogenans*, and performed Reverse Transcription-qPCR. AD_0127 was 20 fold upregulated only on iron citrate conditions. AD_0127 is described as a hypothetical protein, but Pfam predicts it to be C_{554} type cytochrome having a possible role in nitrification (Wang et al., in preparation).

To gain a better understanding of both iron and sulfate reduction among Gram (+) organisms, our focus fell squarely on a versatile microbe, *Desulfotomaculum reducens*. *D. reducens* can grow fermentatively or on sulfate, iron, and also reduces uranium. Through a variety of studies using multidimensional protein-level separations to enrich for iron reducing protein fractions and subsequent heterologous expression (called "top-down" proteomics in this project) we discovered the first two iron reductases in a Gram (+) organism. We also showed that these iron reductases also reduced U and Cr with NADH as the electron donor (Otwell et al., 2015).

We combined our non-denaturing protein separation workflow with functional screening for iron reductase activity ("top-down" protein separations followed by MS-based ID of proteins in active fraction), and heterologous expression of hypothesized iron reductases from multiple study organisms (especially *Desulfotomaculum reducens*, *Geobacter sulfurreducens*, and *Anaeromyxobacter dehalogenans*). Our heterologous expression work gave fundamental insights into the key domains of an iron reductase enzyme with orthologs in *Geobacter sulfurreducens*, *Desulfotomaculum reducens* and other organisms (including groundwater populations at the Rifle, CO, Uranium bioremediation site). This work is published in Li et al., 2015. Additionally, we made methodological contributions to the heterologous expression/cloning community (Kostylev and Otwell et al., 2015).

Given that neither sulfate nor iron reduction enzymes had been confirmed in a Gram (+) organism, we characterized in-depth the global proteomes of *Desulfotomaculum reducens* present under various conditions (HFO, sulfate, fermentation, iron reduction), and used the proteomic data to show that sulfate reduction pathway enzymes (e.g dsrAB) are constitutively expressed (Otwell et al., 2016). Finally, we engineered and constructed a co-culture between *Geobacter sulfurreducens* and *Desulfotomaculum reducens* fed pyruvate as electron donor and soluble iron as electron acceptor. Phenotypically, co-culture growth and iron reduction was faster in the co-culture than in either *Geobacter sulfurreducens* or *Desulfotomaculum reducens* pure cultures alone. Additionally, pyruvate was processed at a faster rate. The interspecies transfer of H_2 and acetate from *Desulfotomaculum reducens* to *Geobacter sulfurreducens* likely played a pivotal role in the enhanced metabolic rates of the co-culture. Geochemical characterization of precipitate formed in the co-culture (absent in the pure cultures) led to the conclusion that both *Desulfotomaculum reducens* and *Geobacter sulfurreducens* contributed to iron reduction, ultimately suggesting that in natural settings multiple different iron reducing populations contribute to reduce iron.

As part of the last phase of this research (Phase 3) we developed targeted quantitative proteomics assays ("MRM" assays) to quantify all known Fe/U reductases. This included those confirmed in the literature as well as those discovered in our current project using "top-down" and "bottom-up" proteomic biomarker discovery (Phases 1&2). We ordered synthetic, isotopically labeled peptides for method development. Mixtures of these isotopically labeled peptides were also used as a spike-in internal standard for analyzing samples. The MRM bioassay was used to look at quantitative changes in key *Desulfotomaculum reducens* and *Geobacter sulfurreducens* proteins during growth in pure cultures versus co-culture conditions.

Summary of Project Activities

Year 1

Phase 1 (Bottom-up proteomics) related activities: *Both in silico and culture-based aspects of Phase 1 were accomplished during this year.*

An in silico comparative genomic comparison of all identified IRB and SRB organisms within the JGI's IMG was undertaken to identify a core genome among each group of electron acceptors. We developed the SPOCs algorithm (Species Pairwise Ortholog Comparisons) to identify orthologous and paralogous genes by a combination of reciprocal best hit and graph theory methods. The traditional graph theory approach to combine the pairwise comparisons from reciprocal best hits is computationally inefficient using a scripting language such as Perl (requiring weeks of compute time for the 33 delta-Proteobacterial species, where there were 528 pairwise comparisons to combine). Our algorithm, using freely available C libraries for graph analysis reduced the compute time for ortholog detection from weeks down to minutes. This tool was published in the highly respected journal *Bioinformatics* (Curtis et al, 2013) and is now available to the public as a web-based tool maintained at PNNL. From our analysis the following was observed:

- 4153 ortholog groups were identified between 12 species of Firmicutes, among which 433 spanned all species. Among the 33 species of delta-Proteobacteria included in our comparison, 219 orthologs were identified in all species, suggesting that with additional species greater convergence on a "core" genome can be achieved. The premised core is hypothesized to represent environment/niche independent functional essentiality, while clusters of orthologs progressing away from the core are more environment/niche dependent essential functions.
- In general, a significant proportion of the total orthologous genes identified (3200 of the 8484) are annotated as hypothetical. This outcome clearly illustrates the challenged faced in systems biology.

The characterization of proteomes for six organisms *Geobacter sulfurreducens (IRB), Geobacter bemidjensis (IRB), Shewanella oneidensis (IRB), Anaeromyxobacter dehalogenans(IRB*), *Desulfotomaculum acetoxidans (SRB)*), and *Desulfotomaculum reducens IRB&SRB*) were begun during year 1 using PNNL's proteomics pipeline. Protein extraction was hampered by the presence of hydrous ferrous oxide (HFO) used as an electron acceptor for the almost all IRB organisms, and Fe-citrate was used as an alternative. Of particular interest was the generation of proteomics data for *D. reducens* because of: 1) its unique status as a Gram (+) metals reducing bacterium, and 2) its dual capability to reduce both iron and sulfate. It should be noted that for several of the organisms selected, this was the first effort towards generation of publicly available proteomics databases.

Phase 2 (Top-Down) related activities

Progress was also made in Year 1 on the development of a high-throughput activity assay for the identification of proteins with iron reduction capability. In our Phase 2 experiments, we proposed to develop a top-down approach for non-denaturing separation of macromolecular complexes into a 96/384-well plate for direct on-plate iron reducing activity assay and subsequent identification of relevant redox enzymes by mass spectrometry for the active

fractions in the plate. We originally tested our non-denaturing, multi-dimensional protein separation workflow on *G. sulfurreducens* in order to develop and optimize this part of the project. Initially gel filtration chromatography on a HiPrep 16/60 Sephacryl S-300 High Resolution column (16 x 600 mm; GE Healthcare) was carried out using AKTA Discovery FPLC system (GE Healthcare) with a fraction collector housing 4 x 96well plates over ice bath. The soluble and insoluble *Geobacter* lysate fractions were combined into a single sample and 15 mg of lysate proteins was injected for isoratic separation of protein complexes. This separation configuration was demonstrated working well for *E coli* lysate in a pilot test case. But we had very limited success for the *G. sulfurreducens* sample, after subsequent activity assay on the 4 x 96-well plates for Fe (III) NTA reduction via production of Fe (II) ferrozine complex by spectrophotometric plate reader. The main bottleneck was a significant loss of enzymatic activity following the above native separation due apparently to 1) long exposure to aerobic atmosphere environment during gel filtration (>10 hours) and 2) interference of phosphate buffer with activity. To overcome these issues, we have applied a recently developed, ultra-high resolution size exclusion column called SEC-3000 from Phenomenex, which was operated using a Dionex UltiMate 3000 HPLC system with the built-in micro fraction collection option in its autosampler and UV detection (Sunnyvale, CA). We have demonstrated that the SEC-3000 column allows faster separation (running cycle down to less than 16min) with very reproducible and high resolution separation of proteins ranged from 5 to 700 kD with theoretical plate number over 50,000.

We successfully used the workflow to suggest the iron reductases in *Anaeromyxobacter dehalogenans* and, for the first time, in a Gram+ iron reducer - *Desulfotomaculum reducens*. Proteins suggested by the top-down assays were selected for heterologous expression in *E. coli* to biochemically prove iron reductase function.

Year 2

Phase 1 (Bottom-up proteomics) related activities:

Both *in silico* and culture-based aspects of Phase 1 continued in year 2. On the *in silico* comparative genomics side of Phase 1, we have mined the literature and genome databases to identify bacterial species with complete genome sequence data available that are capable of iron and/or sulfate reduction. We then identified orthologous genes among the dozens of species by a combination of reciprocal best hit and graph theory methods. We believe that identifying such core genes will highlight oxidoreductases specifically associated with iron and sulfate reduction in known radionuclide-reducing bacteria. We developed a robust software program ("SPOCS") that implements this ortholog prediction approach for any collection of genomes the user desires. We released the software to the community for download and published a corresponding journal article (Curtis et al, 2013):

http://cbb.pnl.gov/portal/software/spocs.html

We have also made the software available as a web service at:

http://cbb.pnl.gov/portal/tools/spocs.html

The SPOCS-output was used, together with proteomics data, to identify putative iron and sulfate oxidoreductases and prioritize genes for down-stream biochemical characterization following heterologous expression (see below).

For the comparative proteomics aspects of Phase 1, we focused on six organisms thus far, including four iron reducers (*Geobacter sulfurreducens, Geobacter bemidjiensis, Shewanella oneidensis,* and *Anaeromyxobacter dehalogenans*), a sulfate reducer (*Desulfotomaculum acetoxidans*), and an organism capable of both respiratory processes (*Desulfotomaculum reducens*). Iron reducing organisms were also cultured on insoluble hydrous ferric oxide (HFO), but technical issues extracting active proteins from these cultures have prevented inclusion of this condition in our comparative proteomic analyses. Oxalate dissolution, acid and temperature were all used to try and improve separation of proteins from minerals in HFO cultures. Though acid extremes could dissolve minerals, iron reductase activity was not retained in the protein pools extracted – suggesting denaturation or protein complex-disassociation.

Phase 2 (Top-Down) related activities

Progress was made in Phase 2 of the project, where the focus is on the identification of proteins/protein complexes capable of reduction through a refined functional assay-based proteome screen in organisms of interest. Separations included anion exchange chromatography (SAX), high resolution size exclusion chromatography (SEC), and native gel electrophoresis. A liquid-based screen for iron reduction activity based on the reagent ferrozine follows SAX and SEC, while an in-gel activity assay is performed following native gel separation. Fractions where iron reduction activity is detected are selected for the next stage of separation. Additionally, we have developed plate reader assays for uranium reduction that can be used to screen for proteins capable of direct U(VI) reduction.

Proteomes investigated through implementation of our optimized workflow include *G. sulfurreducens* grown on fumarate, *D. reducens* grown on sulfate, *D. reducens* grown on Fe(III)-citrate, *A. dehalogenans* grown on fumarate, and *A. dehalogenans* grown on Fe(III)-citrate. Each of these experiments has led to the identification of target proteins for further characterization through heterologous expression. Specifically, our top-down workflow identified three proteins of interest for iron reduction capability in *D. reducens*, and heterologous expression confirmed this predicted function (published in Otwell et al., 2015, *Environmental Microbiology*). These findings represent the first report of specific proteins capable of iron reduction in a Gram-positive organism—a group reported to be environmentally relevant but severely lacking characterization in regards to metal reduction. Similar investigations were made with *A. dehalogenans*, another environmentally relevant organism in which specific iron reduction proteins have not yet been identified. Unfortunately, the cytochromes suggested by the top-down discovery approach were not able to be functionally expressed heterologously despite coexpression of enzymes involved in cytochrome assembly. The full list of targets for heterologous expression that were selected from analyses of *A. dehalogenans,G. sulfurreducens* and *D. reducens*, (Tables 1, 2 & 3 below).

Tables 1-3: Genes cloned for biochemical analyses of discovered oxidoreductases putatively involved in iron or sulfate reduction pathways. ***Bold italicized text*** highlights proteins confirmed to have iron reductase activity following heterologous expression

Table 1. Heterologous Characterization Progress for *Desulfotomaculum reducens* MI-1

Protein ID	Annotation	Current Status	Iron(III) Reduction Activity

Dred_0047	Pyruvate flavodoxin/ferredoxin oxidoreductase domain-containing protein (alpha subunit)	Purified	No
Dred_1340	Hypothetical protein	Overexpressed	No
Dred_1341	Dinitrogenase iron-molybdenum cofactor biosynthesis	Overexpressed	No
Dred_1445	Nitrate reductase subunit gamma	Overexpressed	No
Dred_1446	Hypothetical protein	Overexpressed	No
Dred_1685	Oxidoreductase FAD/NAD(P)-binding subunit	Purified	Yes
Dred_1686	***Dihydroorotate dehydrogenase 1B***	***Purified***	***Yes (NADPH-dependent)***
Dred_2273	Aldehyde ferredoxin oxidoreductase	Purified	No
Dred_2421	***NADH:flavin oxidoreductase***	***Purified***	***Yes (NADH-dependent)***

Table 2. Heterologous Characterization Progress for *Geobacter sulfurreducens* PCA

Protein ID	Annotation	Current Status	Iron(III) Reduction Activity
GSU0216	Hypothetical protein	Purified	No
GSU0217	Nitroreductase family protein	Purified	No
GSU0476	Hypothetical protein	Purified	No
GSU1467	Iron-sulfur cluster-binding protein	Purified	No
GSU1371	***NADPH-dependent enal/enone/nitroreductase***	***Purified***	***Yes (NADH- or NADPH-dependent)***
GSU1468	2-oxoglutarate ferredoxin oxidoreductase subunit alpha	Purified	No
GSU1469	2-oxoglutarate ferredoxin oxidoreductase subunit beta	Purified	No
GSU1470	Keto/oxoacid ferredoxin oxidoreductase, subunit gamma	Purified	No
GSU1467-1470	***2-oxoglutarate ferredoxin oxidoreductase complex***	***Purified***	***Yes [Independent of NAD(P)H]***
GSU2564	Hypothetical protein	Purified	No

Table 3. Heterologous Characterization Progress for *Anaeromyxobacter dehalogenans* 2CP-1

Protein ID	Annotation	Current Status	Iron(III) Reduction Activity
A2cp1_0127	Hypothetical protein (Cytochrome_C554; Cytochrom_B_N)	Cloned; formed inclusion	Not determined

		bodies	
A2cp1_0241	Pyruvate flavodoxin/ferredoxin oxidoreductase domain-containing protein	Purified	No
A2cp1_1731	Multiheme cytochrome	Cloned; formed inclusion bodies	Not determined

In addition to confirming the function of novel iron reductases in *D. reducens*, we examined the specific domains involved in iron reduction in one of these iron reductases (ultimately published in Li et al, 2015). We also collaborated with the Yo Suzuki lab at the Craig Ventner Institute to develop a novel cloning method (ultimately published in Kostylev et al. 2015).

Year 3 +No-Cost Extension period

In Year 3 and the no cost extension period, we extended our studies on *Desulfotomaculum reducens* to include proteomic comparisons of this versatile microorganism on sulfate, iron (soluble), insoluble iron (Hydrated-ferric oxide – HFO), pyruvate fermentation, and in co-culture with a model iron reducer, *Geobacter sulfurreducens*. We selected growth conditions where *Geobacter sulfurreducens* relies upon *Desulfotomaculum reducens* for electron donor (H_2 and acetate) and both organisms contribute to iron reduction. For the comparative proteomics work in these studies the Accurate Mass and Time tag (AMT) approach commonly used by the EMSL facility at PNNL was taken. Highlights from the *Desulfotomaculum reducens* pure culture studies are published in Otwell 2016 (Frontiers of Microbiology) and a forthcoming manuscript (Otwell in preparation) and include:

1. Successful proteome characterization of cultures grown on insoluble iron precipitates (which was not successful earlier in the project) and evidence that the sole cytochrome in *Desulfotomaculum reducens* is indeed expressed on insoluble iron.
2. *Desulfotomaculum reducens* expressed most of its predicted sulfate reduction pathway enzymes REGARDLESS of whether sulfate is present – suggesting that expression may be constitutive.
3. Evidence for flavin-based electron bifurcation in *Desulfotomaculum reducens*
4. Accelerated Fe-reduction and pyruvate conversion rates in co-cultures of *Desulfotomaculum reducens* and *Geobacter sulfurreducens* compared to either culture in isolation. Both organisms contribute to Fe-reduction and the *Desulfotomaculum reducens*-specific iron mineral (vivianite) was heavily colonized by *Geobacter* cells during growth.

In this period of work we also developed and deployed a novel method for quantitative specific detection of all documented iron and uranium reductases (from gamma-proteobacteria, delta-proteobacteria, and Firmicutes) including those discovered in Phase 2 Top-down enzyme discovery studies. We ordered synthetic isotopically labeled versions of "proteotypic" peptides unique to each protein of interest and developed a MS-based method for Multiple Reaction Monitoring (MRM) assays. Some hurdles were encountered with some of the peptides during the validation step: where some peptides could not be validated in existing cultures nor Rifle groundwater biomass samples. Nonetheless, the developed method could be repeated by other

researchers on lab and environmental samples. We deployed the MRM assay on the pure and co-culture biomass of *Geobacter sulfurreducens* and *Desulfotomaculum reducens.* The generation of both shotgun (AMT tag) data and MRM data also allowed us to show that the relative trends in protein quantities determined by the two methods were in general agreement. These results are included in a PhD dissertation (Otwell PhD, 2016) and are being prepared in an additional manuscript.

With respect to metaanalyses of the collective bottom up proteomic datasets, a MS thesis and upcoming manuscript overlayed protein expression trends over SPOCS-identified orthologous clusters for the four proteobacteria, *Geobacter sulfurreducens, Geobacter bemidjiensis, Anaeromyxobacter dehalogenans,* and *Shewanella oniedensis.* For each culture proteomes from Fe-citrate (soluble iron) grown cells were compared to those on fumarate. We focused on the orthologous clusters that consistently (across organisms) showed upregulation on iron compared to fumarate. The most notable consistent upregulation was of membrane-bound ATPase complex subunits. This could be an effect of the greater energy available for conserving when iron rather than fumarate is the electron acceptor. Of these organisms, *Anaeromyxobacter dehalogenans,* has the least understood iron reduction pathways. Our metaanalysis of shotgun data combined with the results from the Top-down assays provide strong evidence that two of the dozens of cytrochromes are upregulated on iron: Adeh_0127 and Adeh_1731. These trends were confirmed at the transcript (Wang MS Thesis 2015, Wang et al, in preparation).

Products of Award:

Peer-reviewed Journal Articles:

1. Otwell, A.E., Callister, S.J., Zink, E.M., Smith, R.D., Richardson, R.E. (2016) Comparative Proteomic Analysis of *Desulfotomaculum reducens* MI-1: Insights into the Metabolic Versatility of a Gram-positive Sulfate and Metal-reducing Bacterium. Frontiers in Microbiology. doi:10.3389/fmicb.2016.00191
2. Li, Z., Kim, D.D., Nelson, O.D., Otwell, A.E., Richardson, R.E., Callister, S.J., Lin, H. (2015) Molecular dissection of a putative iron reductase from *Desulfotomaculum reducens* MI-1. Biochemical and Biophysical Research Communications 467, 503–508. doi:10.1016/j.bbrc.2015.10.016
3. Kostylev, M., Otwell, A.E., Richardson, R.E., Suzuki, Y. (2015) Cloning Should Be Simple: *Escherichia coli* DH5α-Mediated Assembly of Multiple DNA Fragments with Short End Homologies. PLOS ONE 10, e0137466. doi:10.1371/journal.pone.0137466
4. Otwell, A.E., Sherwood, R.W., Zhang, S., Nelson, O.D., Li, Z., Lin, H., Callister, S.J., Richardson, R.E. (2015) Identification of proteins capable of metal reduction from the proteome of the Gram-positive bacterium *Desulfotomaculum reducens* MI-1 using an NADH-based activity assay: Metal reductases from Gram-positive metal reducer. Environmental Microbiology 17, 1977–1990. doi:10.1111/1462-2920.12673
5. Curtis, D.S., Phillips, A.R., Callister, S.J., Conlan, S., McCue, L.A. (2013) SPOCS: software for predicting and visualizing orthology/paralogy relationships among genomes. Bioinformatics 29, 2641–2642. doi:10.1093/bioinformatics/btt454

Manuscripts in preparation:

1. Otwell, Anne E., Stephen J. Callister, Robert W. Sherwood, Sheng Zhang, Ruth E. Richardson. Phenotypic and proteomic analysis of an Fe(III)-reducing co-culture established

between *Geobacter sulfurreducens* and *Desulfotomaculum reducens* (to be submitted March 2016)
2. Wang, Qiaochu, Otwell, Anne E., Callister, Stephen J., and Richardson, Ruth E. Proteome shifts in *Anaeromyxobacter dehalogenans* during growth on iron compared to model Gram-negative iron reducing organisms: implications for iron reducing cytochromes in non-model Gram-negative iron-reducing bacteria. (to be submitted April 2016).

Theses:
1. Otwell,PhD. 2016. PhD Thesis. March 2016." Insights into microbial metal reduction through implementation of proteomic-based techniques: A focus on the Gram-positive bacterium *Desulfotomaculum reducens* MI-1"

2. Wang, Qiaochu. 2015. MS Thesis. August 2015. "Conserved and specific proteome responses to growth on ferric citrate versus fumarate in various Gram (-) dissimilatory iron reducing bacteria: *Anaeromyxobacter dehalogenans* 2CP-1, *Shewanella oneiden*sis MR-1, *Geobacter sulfurreducens* PCA AND *Geobacter bemidjiensis* Bem"

Undergraduate research projects:
1. Hernsdorf, Alex. B.S. 2014 (currently Graduate student at UC Berkeley in the Banfield lab). "New Analytical Tools for Inter- and Intraspecies Proteogenomic Comparisons" and "Software Tools for Proteotypic Peptide Selection" (two projects)
2. Hartleb, Marissa. B.S. 2014 (currently Graduate student at Penn State University) "mRNA Comparative Analysis of Desulfotomaculum reducens MI-1 with Various Electron Acceptors"

Graduate Student Rotation Project
1. St.James, Andrew. 2015. "Evidence of increased expression of two putative iron reductases in *Anaeromyxobacter dehalogenans* strain 2CP-1 when grown with ferric iron as electron acceptor.

Conference Abstracts/Poster Presentations:
1. Otwell AE, Sherwood RW, Nelson OD, Li Z, Lin H, Callister SJ, Richardson RE, Zhang S. 2014. A function-based intact protein analysis workflow for discovery of novel microbial metal and uranium reductases in a Gram positive bacterium. Proceedings of the 62nd ASMS Conference on Mass Spectrometry and Allied Topics. Baltimore, MD. June 15-19, 2014. Poster #: TP203.
2. Otwell, A.E., Sherwood, R.W., Zhang, S., Nelson, O.D., Lin, H., Callister, S.J., Richardson, R.E., 2014. Discovery of Metal Reductases in the Versatile Gram-positive Bacterium Desulfotomaculum reducens MI-1 Using Varied Proteomic Techniques. Abstracts of the General Meeting of the American Society for Microbiology 114, 1886–1886.
3. Callister, S.J., Otwell, A.E., McCue, L., Wilkins, M.J., Sherwood, R.E., Curtis, D.S., Phillips, A.R., Zhang, S., Lin, H., Richardson, R.E., 2013. From Biological Process to Protein Function: Understanding Microbial Iron and Sulfate Reduction through Functional Proteomics Assays. Abstracts of the General Meeting of the American Society for Microbiology 113, 271–271.

4. Otwell, Anne E DOE Conference “Investigations into the proteome of Gram-positive metal-reducing bacterium *Desulfotomaculum reducens* MI-1” February 2014

5. Nash, Ornella. Identification of Iron Reductases Using Top-down Proteomics and Heterologous Expression” - Poster presentation at U.S. Department of Energy Office of Science 2014 Genomic Science Contractor-Grantee Meeting XII. 02/2014
6. Nash, Ornella, Identification of Iron Reductases Using Top-down Proteomics and Heterologous Expression” - Poster presentation at Cornell University Chemistry Biology Interface (CBI) Annual Symposium. 04/2014
7. Otwell, Anne E DOE Conference “Improved Understanding of Microbial Iron and Sulfate Reduction Through a Combination of Bottom-up and Top-down Functional Proteomic Assays”, February 2013

8. Otwell, Anne E Northeastern Microbiology’s: Physiology, Ecology, and Taxonomy Conference (NMPET) “Improved understanding of microbial iron reduction through a proteomic-based high-throughput activity assay”, June 2012

Conference talks:

1. Otwell, Anne E (PhD student), Gordon Research Seminar, Applied and Environmental Microbiology, 2015. “Insights into microbial metal reduction using varied proteomic techniques: a focus on the Gram-positive bacterium *Desulfotomaculum reducens* MI-1.”

2. Richardson, Ruth E. U.S. Department of Energy Office of Science 2014 Genomic Science Contractor-Grantee Meeting XII , 2014.” Application of proteomics to understand microbial iron and sulfate reduction”