Metaproteomics of Subsurface Microbial Communities at Contaminated DOE Sites

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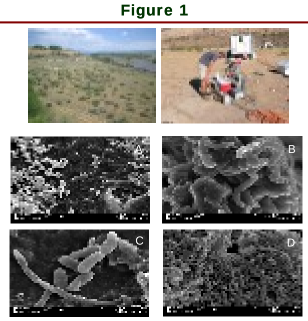
OVERVIEW

- The *Geobacter* species are one of the most abundant and highly characterized microbial species recognized in the soil microbiome. The characterization of the species has revealed multiple biological capabilities shown to be of high environmental significance. The anaerobic respiring bacterial species are capable of completely oxidizing organic matter to carbon dioxide to allow electrons to be transferred to acceptors such as Fe (III), toxic metals, humic substances, and electrodes. These physiological characteristics make the *Geobacter* species an important agent in the bioremediation of contaminated soils and groundwater (Lovely, *BBA* 2006).
- Subsurface microbial communities that are located at contaminated DOE sites actively alter the geochemical properties of metal-contaminated subsurface environments. By studying functional gene expression of the complex bacterial ecosystem, a potential remediation strategy for metal-contaminated subsurface environments can be deployment of microbes for biotransformation of the oxidized, mobile metals to sparingly soluble, reduced forms can be developed by characterizing the metaproteome.

Approach

- Success of such a strategy depends on knowledge of structure and dynamics of the indigenously active microbial community. Metaproteomic techniques have tremendous potential in unraveling the molecular level protein details of active *in situ* microbial communities in soils or ground-waters. To understand the complex bacterial communities, shotgun MS proteomic measurements were employed on three ground-water field samples, effluent column tubing, and metal contaminated soil in the Rifle Field site in Colorado (Figure 1).
- Our methodology for the metaproteome analyses involved sample collection (~500L of groundwater), via tangential flow filtration, centrifugation, single tube cell lyses, protein denaturation and protease digestion with trypsin followed by automated analysis with 2-dimensional nano-LC-MS/MS on a LTQ-Orbitrap (Figure 2).
- The goal of this study was to use MS-based proteomics to characterize the metabolic potential and biological interactions occurring in a metal-contaminated site.

Figure 1



Images A - D: Electron microscope screening capturing microbial species found in the effluent column tubing. A variety of species were found; however, the tubing was heavily dominated by *Dechloromonas aromatica*

METHODS

- We applied 2D-LC-MS/MS for the characterization of microbial communities collected from ground-water, column tubing, and soil sediments found within the DOE site in Rifle Colorado.
- The column tubing collected was sliced and placed in a buffer to allow a vigorous vortex to release all microbes into solution, and then processed through ORNL proteomics pipeline.
- Three spatial resolved samples with 50 mLs of concentrated water from ~500 L were collected from Rifle field site for metagenomics and metaproteomics (Figure 3). The flow-through, containing the cells, was concentrated using tangential flow filtration (TFF) followed by centrifugation where approximately 1 mL was removed for sample processing.
- The microbial pellets were lysed via a single tube small processing method, and the proteins were then denatured, reduced, and digested with trypsin. Samples were analyzed with technical duplicates via two-dimensional (2D) nano-LC MS/MS system with a split-phase column (RP-SCX-RP) on a hybrid linear ion trap-Orbitrap (Thermo Finnigan). Full scans were acquired at 30,000 resolution in the Orbitrap; MS/MS scans were acquired in the LTQ (Figure 2).
- All samples were searched against a database that contained ~26,000 Geobacter proteins (seven species) and a total of ~3 million microbial proteins from 862 different species.
- All MS/MS spectra were searched with the SEQUEST algorithm, against database above, and filtered with DTASelect/Contrast at the peptide level with conservative filters [Xcorrs of at least 1.8 (+1), 2.5 (+2) 3.5 (+3)]. Only proteins identified with two fully tryptic peptides at conservative filters were considered for further biological study.

Figure 2

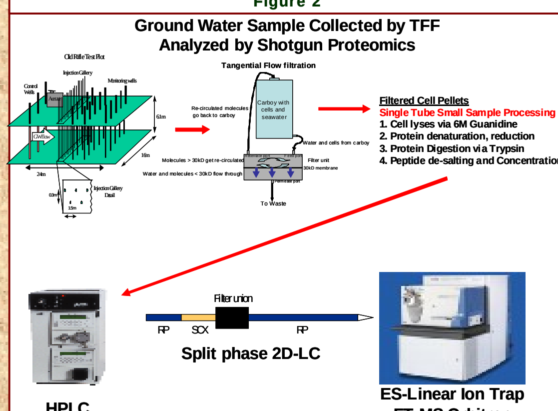
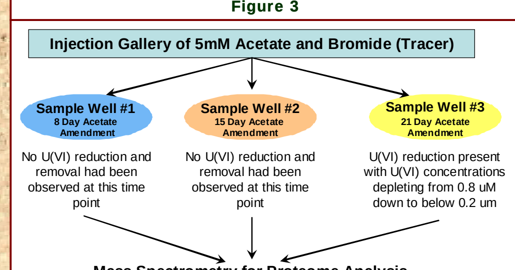


Figure 3



RESULTS AND DISCUSSION

Global Results

- In this study, we extracted microbial communities from ground-water field samples, effluent column tubing, and metal contaminated soil.
- The complex peptides mixtures were run by 2D-LC-MS/MS on a LTQ-Orbitrap and the chromatograms were very complex, with 100,000s of peptides.
- Three different acetate amended ground-water sites that were spatially resolved were sampled to investigate the microbial community composition at a strain level and also protein expression to elucidate a metabolic signature.
- We have obtained deep proteomes (~3,000 proteins) that were heavily dominated by *Geobacter* species from ground water samples, which provided information about *Geobacter* the physiology during uranium reduction after acetate amendment.
- The observed *Geobacter* proteins were what one would expect from a proteome, such as the highly abundant proteins involved in TCA, translation, nitrogen fixation, and flagella/chemotaxis.
- All three sample sets contained different species distribution profiles. Table 1 illustrates the variability that was found in species abundance by providing a list of the total unique spectra, and non-unique spectra from each database entry type.
- The effluent column tubing contained a more complex microbial community than the ground-water community, but was dominated by a single bacterial type best matching to *Dechloromonas aromatica,* with ~500 proteins identified with many unique spectra.
- From the column tubing, the most abundant protein was phasin and is specific to *Dechloromonas aromatica*. Out of four analyses, it was found with high estimated abundance by 2 orders on average (Table 3).

Table 1

Sample-Set1(Run1) Species	Unique count
Geobacter M21	741
Geobacter bemidjiensis	394
Geobacter uraniumreducens RF4	378
Geobacter sp. FRC-32	354
Geobacter sulfurreducens	204
Geobacter lovleyi SZ	196
Geobacter metallireducens GS-15	160

Sample-Set1(Run2) Species	Unique count
Geobacter M21	820
Geobacter bemidjiensis	434
Geobacter uraniumreducens RF4	392
Geobacter sp. FRC-32	380
Geobacter sulfurreducens	235
Geobacter lovleyi SZ	238
Geobacter metallireducens GS-15	181

Sample-Set1(Run3) Species	Unique count
Geobacter M21	790
Geobacter bemidjiensis	395
Geobacter uraniumreducens RF4	396
Geobacter sp. FRC-32	393
Geobacter sulfurreducens	233
Geobacter lovleyi SZ	192
Geobacter metallireducens GS-15	166

Sample-Set2(Run1) Species	Unique count
Geobacter M21	894
Geobacter bemidjiensis	451
Geobacter uraniumreducens RF4	580
Geobacter sp. FRC-32	336
Geobacter sulfurreducens	263
Geobacter lovleyi SZ	212
Geobacter metallireducens GS-15	176

Sample-Set2(Run2) Species	Unique count
Geobacter M21	844
Geobacter bemidjiensis	446
Geobacter uraniumreducens RF4	590
Geobacter sp. FRC-32	273
Geobacter sulfurreducens	245
Geobacter lovleyi SZ	196
Geobacter metallireducens GS-15	162

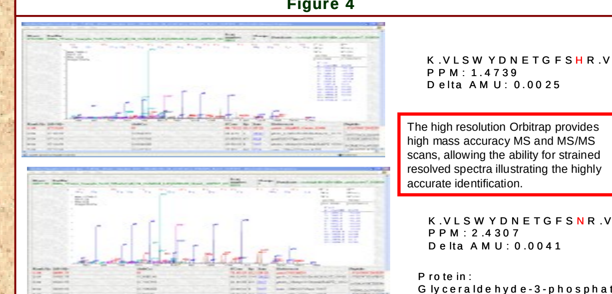
Sample-Set2(Run3) Species	Unique count
Geobacter M21	817
Geobacter bemidjiensis	402
Geobacter uraniumreducens RF4	519
Geobacter sp. FRC-32	289
Geobacter sulfurreducens	269
Geobacter lovleyi SZ	198
Geobacter metallireducens GS-15	170

Sample-Set3(Run1) Species	Unique count
Geobacter M21	643
Geobacter bemidjiensis	296
Geobacter uraniumreducens RF4	517
Geobacter sp. FRC-32	313
Geobacter sulfurreducens	273
Geobacter lovleyi SZ	510
Geobacter metallireducens GS-15	217

Sample-Set3(Run2) Species	Unique count
Geobacter M21	559
Geobacter bemidjiensis	233
Geobacter uraniumreducens RF4	502
Geobacter sp. FRC-32	276
Geobacter sulfurreducens	209
Geobacter lovleyi SZ	438
Geobacter metallireducens GS-15	202

Sample-Set3(Run3) Species	Unique count
Geobacter M21	677
Geobacter bemidjiensis	263
Geobacter uraniumreducens RF4	500
Geobacter sp. FRC-32	297
Geobacter sulfurreducens	293
Geobacter lovleyi SZ	471
Geobacter metallireducens GS-15	216

Figure 4



The high resolution Orbitrap provides high mass accuracy MS and MS/MS scans, allowing the ability for strained resolved spectra illustrating the highly accurate identification.

Table 2

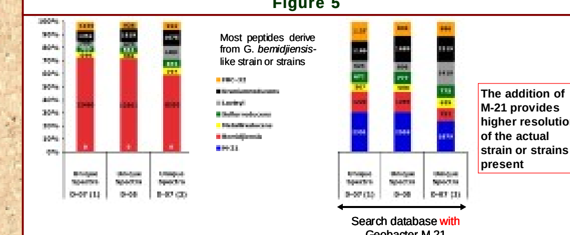
	M-21	Bemidjiensis	Metallireducens	Sulfurreducens	Uraniireducens	FRC-32	Lovleyi
M-21	-	94%	65%	64%	67%	66%	60%
Bemidjiensis	94 %	-	65%	64%	67%	66%	60%
Metallireducens	65 %	65%	-	76%	68%	67%	62%
Sulfurreducens	64 %	64%	76%	-	67%	66%	62%
Uraniireducens	67 %	67%	68%	67%	-	74%	61%
FRC-32	66 %	66%	67%	66%	74%	-	61%
Lovleyi	60 %	60%	62%	62%	61%	61%	-

Average ortholog % aa similarity

Detection of Strain Variation in a Complex Microbial Community

- The high resolution Orbitrap provides high mass accuracy that will allow microbial composition to be determined at the strain level. The ability to identify a single amino acid changes in an amino acid sequence makes differentiation between different strains possible (Figure 4). Therefore, the investigation into whether communities are dominated by one strain / species or several is achievable (Figure 5).
- The addition of M-21 strain into the database provides higher resolution of the actual strain or strains present. This is due to their orthogonal relationship as seen in Table 2. In theory, all unique spectra could be generated by one *Geobacter* species or derive from multiple species (Figure 5). For example, following a mutation event the peptides will obtain a new mass; this change is readily detectable via mass spectrometry (Figure 6).
- The ability for quantitative measurements between closely related starines to deterimine which isolate is the dominant strain/species or most closely related to is shown in Figure 7. The conserved non-unique peptides can serve as a measure of changes in strain/species abundance in the community. The identification of multiple unique spectra also indicates multiple coexisting strains are present.

Figure 5



The addition of M-21 provides higher resolution of the actual strain or strains present

Figure 6

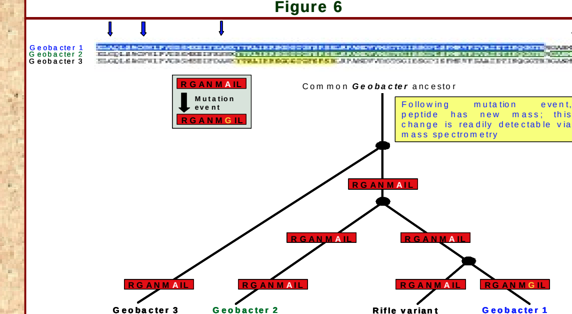
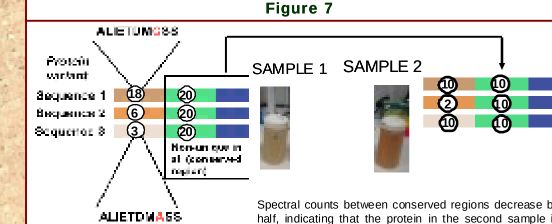


Figure 7

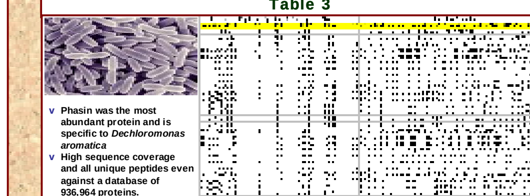


Spectral counts between conserved regions decrease by half, indicating that the protein in the second sample is less abundant

The Metabolic Profile in Metal-Contaminated Ground-Water

- The abundant *Geobacter* proteins observed were those commonly required for translation, the TCA cycle (thus metabolically active), chaperones, nitrogen fixation, and flagella/chemotaxis. The *Geobacter* strains were dominated by Gbem / M21-like species.
- There were multiple unique peptides for TCA cycle, ATP synthase, and NADH dehydrogenase proteins, which indicate activity of multiple *Geobacter* species.
- The transformation in protein abundance levels over time indicates changes in species roles in the community (activity or number) as seen in Figure 9.
- Figure 10 illustrates the expression of proteins involved in nitrogen fixation in all three samples by multiple co-existing *Geobacter* species. Peptide mapping indicates that abundance for the nitrogenase β chain is similar in D-07(1) and D-07(2), samples from early and mid biostimulation.
- During the experiment, changes in the abundances of proteins involved in chemotaxis suggest that *Geobacter* species rapidly respond to changing in environmental stimuli.

Table 3



- Phasin was the most abundant protein and is specific to *Dechloromonas aromatica*
- High sequence coverage and all unique peptides even against a database of 936,964 proteins.

Figure 9

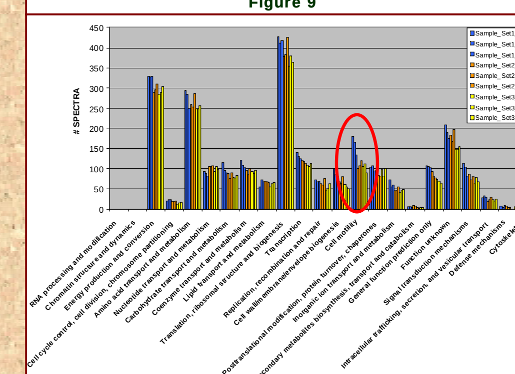
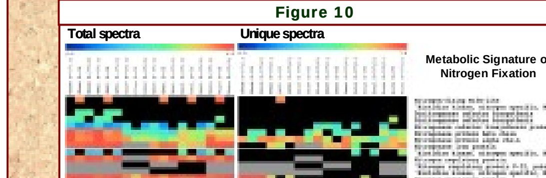


Figure 10



Soil Proteomics

- Extraction of microbial proteins from low biomass soil samples in the presence of deterrents such as humic acid poses quite a distinct challenge. We have compared several *in situ* lysis methods for efficient extraction of proteomes from various metal-contaminated sediment samples. Various methods of pre-treating the soils prior to microbial lysis gave enhanced protein yields.
- In these methods, we were capable of obtaining over 100 proteins, primarily from the dominant organisms.
- *P. putida* F1 is being used as our test organism to evaluate seeding and nutritional amendment effects on microbial community dynamics and concomitant Cr(VI) reduction.
- We have investigated how *Pseudomonas putida* strain F1 responds to a chromate challenge. Of the ~1700 proteins identified by MS, about 100 were upregulated under chromate shock.

CONCLUSIONS

- We have shown that "shotgun" proteomics can be applied to the complex microbial communities seen in ground-water field samples and effluent column tubing from metal-contaminated subsurface environments in the Rifle Field site in Colorado.
- The high mass accuracy community proteomic data provided by the Orbitrap gave insight into changes in both microbial composition at the strain level and the metabolic signature as a result of variations in the geochemical environment.
- Our future goals for soil proteomics would be to increase the identified protein number ten-fold. The use of shotgun MS proteomics will provide the necessary insight into the proteomic profiles of the soil microbes, providing information about functional activities in these environmental communities.

ACKNOWLEDGMENTS

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