USE OF METAGENOMIC AND META-TRANSCRIPTOMIC ANALYSIS TO INTERPRET BIOGEOCHEMICAL PROCESSES MEDIATED BY HANFORD 100H AQUIFER BACTERIA (LAB AND FIELD STUDIES)

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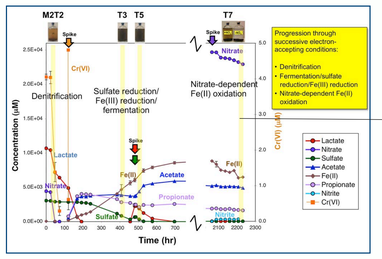
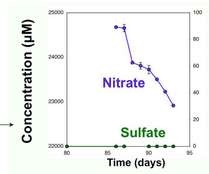
ABSTRACT

We are developing the use of biomolecular signatures as part of the LBNL SFA challenge entitled "Unraveling Biogeochemical Reaction Networks Mediating Sustained Chromium Reduction", which focuses on *in situ* reductive immobilization of Cr at DOE's Hanford 100H site. By applying a high-throughput approach that uses metagenomic and meta-transcriptomic data, we hope to identify highly expressed genes in a specific microbial community under conditions of interest, without requiring any *a priori* sequence information or assumptions about what processes might be occurring. A key scientific goal is to determine if there are diagnostic biomolecular signatures indicative of important aquifer biogeochemical processes that can be used to (a) help discriminate between direct (enzymatic) and indirect (abiotic) oxidation-reduction processes relevant to bioremediation and (b) to inform and constrain reactive transport models even when geochemical field measurements do not reveal all relevant processes. We have collected metagenome and meta-transcriptome sequence information from various experimental systems (including flow-through columns and batch microcosms inoculated with Hanford aquifer sediment or groundwater, bacterial isolates from the flow-through columns, and site groundwater) under conditions relevant to *in situ* chromate reduction and re-oxidation at Hanford 100H. Overall, 12 metagenome and 14 meta-transcriptome samples were sequenced, ranging from 0.1 to 1.6 Gb of sequence data per sample. This poster gives an overview of several noteworthy findings from these Hanford studies.

HANFORD MICROCOSMS: Meta-transcriptomics reveals geochemically undetected microbial process

In microcosm studies designed to characterize functional changes in an aquifer-derived, chromate-reducing microbial community as it transitions successively through electron-accepting conditions relevant to the Hanford subsurface, one condition tested was nitrate-dependent oxidation of Fe(II) and reduced S compounds ("T7"). Meta-transcriptome sequence data revealed expression of nitrate reductase (*nar*) and S oxidation (*sox*) gene clusters from an alpha-Proteobacterial species, whereas routine geochemical (ion chromatography) data did not yield evidence of the S oxidation product. Thus, the meta-transcriptomic data provided insights into biogeochemical processes that would not have been detected by routine geochemical analysis.



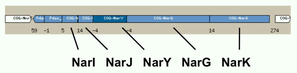


Nitrate-dependent sulfide oxidation

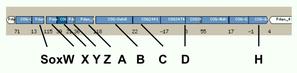
$HS^- + 1.6\ NO_3^- + 0.6\ H^+ \rightarrow SO_4^{2-} + 0.8\ N_2 + 0.8\ H_2O$

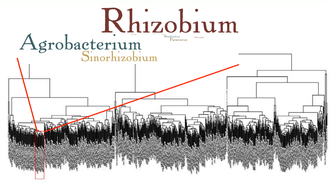
Meta-transcriptome data revealed expression of gene clusters for nitrate reduction and S oxidation (apparently from the same α-Proteobacterial species)

Nitrate reduction (*nar*) gene cluster



S oxidation (*sox*) gene cluster





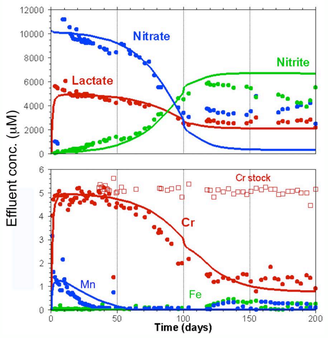
Tetranucleotide frequency (TNF) clustering of T7 assembled contigs > 5kb. The red-outlined cluster contains contigs with almost identical TNFs and coverage (13.4X) indicating a common origin. These contigs contain >700 ORFs with almost 750 kb and include full nitrate reduction and reduced sulfur oxidation operons. Phylogenetically they are affiliated with alpha-proteobacteria, mostly Rhizobium. Meta-transcriptome mapping shows that the complete nitrate reduction (*nar*) and sulfur oxidation (*sox*) operons of the Rhizobium-like species was expressed in the T7 meta-transcriptome. Sulfur oxidation was not apparent from the geochemical measurements.

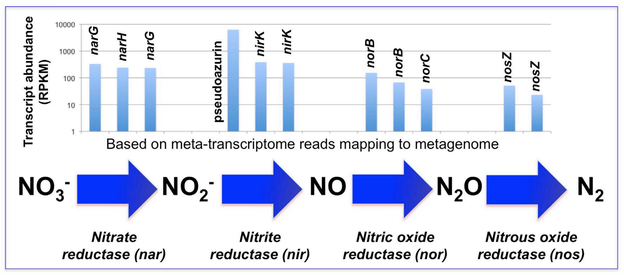
SEQUENCE DATA FROM HANFORD LAB AND FIELD STUDIES

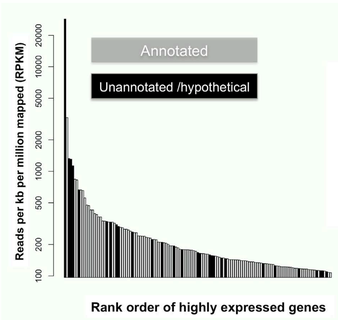
System	Name	Physiology	Sequence type	Reads after QC	Bp after QC
Microcosm	M2T2	Denitrifying	Metagenome	1.00E+07	5.01E+08
	M2T2	Denitrifying	Metatranscriptome	1.95E+06	7.95E+07
	T3	Fermenting/sulfate reduction	Metagenome	1.12E+07	5.64E+08
	T3	Fermenting/sulfate reduction	Metatranscriptome	2.44E+07	1.20E+09
	T5	Sulfate/iron reduction	Metagenome	1.06E+07	5.32E+08
	T5	Sulfate/iron reduction	Metatranscriptome	2.55E+07	1.26E+09
	T7	Nitrate-dependent iron/sulfur oxidation	Metagenome	1.15E+07	5.79E+08
	T7	Nitrate-dependent iron/sulfur oxidation	Metatranscriptome	2.12E+07	1.05E+09
Flow through columns	N5-3B	Denitrifying	Metagenome	3.55E+07	2.75E+09
	S5-5B	Fermenting	Metagenome	1.87E+07	1.43E+09
	S5-5B	Fermenting	Metatranscriptome	6.39E+06	3.16E+08
	Col2	Denitrifying	Metatranscriptome	6.24E+06	3.04E+08
	Col3	Denitrifying	Metatranscriptome	3.02E+07	1.48E+09
	Col10	Denitrifying	Metatranscriptome	9.90E+06	4.81E+08
	Col11	Denitrifying	Metatranscriptome	2.04E+04	9.91E+05
Groundwater from Hanford 100H	PreInjection	Before lactate injection	Metagenome	1.56E+07	1.17E+09
	PreInjection	Before lactate injection	Metatranscriptome	5.97E+06	2.95E+08
	Day2	Denitrifying	Metagenome	1.30E+07	1.30E+09
	Day2	Denitrifying	Metatranscriptome	3.11E+07	1.54E+09
	Day4	Fermenting/Sulfate/Iron reduction	Metagenome	5.34E+06	3.98E+08
	Day4	Fermenting/Sulfate/Iron reduction	Metatranscriptome	1.27E+07	6.27E+08
	Day7	Fermenting/Sulfate/Iron reduction	Metagenome	1.28E+07	1.15E+09
	Day7	Fermenting/Sulfate/Iron reduction	Metatranscriptome	1.66E+07	8.21E+08
	Day11	Lactate depletion/re-oxidation	Metagenome	2.16E+06	1.90E+08
	Day11	Lactate depletion/re-oxidation	Metatranscriptome	3.63E+07	9.07E+08
	Day17	Lactate depletion/re-oxidation	Metagenome	5.59E+05	4.16E+07
	Day17	Lactate depletion/re-oxidation	Metatranscriptome	2.05E+07	9.95E+08
Totals				**3.96E+08**	**2.20E+10**

HANFORD COLUMN META-TRANSCRIPTOMES: Highly expressed "hypotheticals" detected

In flow-through columns containing Hanford aquifer sediment and reducing Cr(VI) under denitrifying conditions, meta-transcriptome data revealed complete denitrification (with expression of *nar*, *nir*, *nor*, and *nos* genes, as well as the highly expressed pseudoazurin), but the most highly expressed genes encoded proteins of unknown function. This finding stresses an advantage of metagenomic and meta-transcriptomic sequencing over *a priori* approaches, namely, that sequencing can reveal important but unannotated genes/enzymes and focus research on such knowledge gaps.



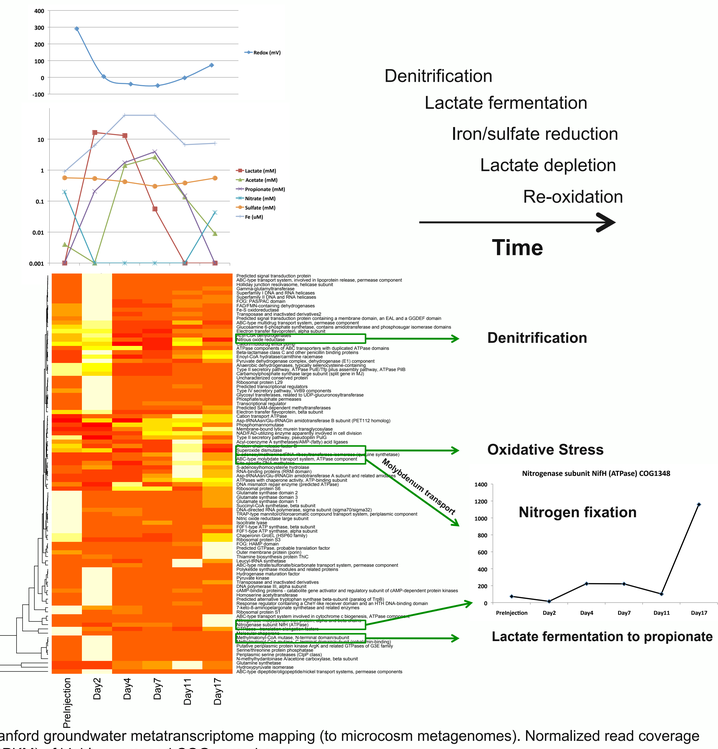




Rank ordering of transcripts from a denitrifying column mapping to the column metagenome reveals that transcripts encoding proteins of unknown function are among the most highly expressed. This has been observed in a large percentage of environmental meta-transcriptome studies published to date, and suggests that *a priori* approaches to characterizing microbial community gene expression may be inadequate.

HANFORD GROUNDWATER SAMPLES: Meta-transcriptomes reveal *in-situ* conditions and metabolism (such as N limitation / N_2 fixation)

Metagenome and meta-transcriptome sequencing was carried out on groundwater samples collected during various biogeochemical regimes (e.g., denitrification, iron/sulfate reduction) that followed lactate biostimulation at the 100H site. Among other findings was evidence of nitrogen limitation, which provided information on amendments needed to optimize *in situ* biostimulation.



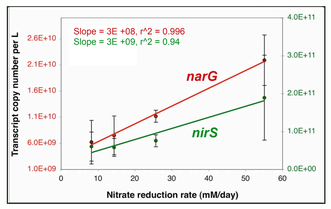
Hanford groundwater metatranscriptome mapping (to microcosm metagenomes). Normalized read coverage (RPKM) of highly expressed COGs are shown.

FUTURE DIRECTIONS

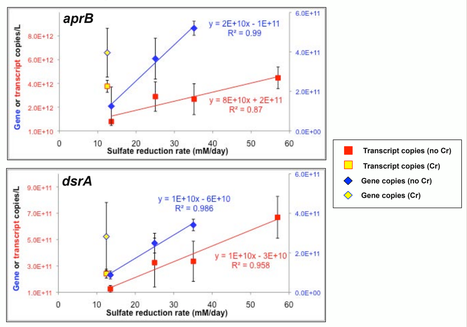
Following this biomolecular signature discovery phase that involves metagenome and meta-transcriptome sequencing of a range of Hanford-relevant biogeochemical conditions, we are developing **meta-transcriptome-based gene expression microarrays** that we hope will allow us to rapidly profile the *in situ* gene expression of Hanford microbial communities on a qualitative basis (i.e., what pathways are most highly expressed?) and a semi-quantitative basis (using activity-signature relationships, see below).

We have already begun to develop metabolic **activity-signature relationships** for several diagnostic genes (e.g., *narG* and *nirS* for denitrification; *dsrA* and *aprB* for sulfate reduction) to relate gene or transcript copy number to the metabolic rate of the relevant process. The data below were generated in chemostat experiments with Hanford isolates: denitrifying *Pseudomonas stutzeri* strain RCH2 and sulfate-reducing *Desulfovibrio vulgaris* strain RCH1.

Activity-signature relationships



Hanford strain RCH2



Hanford strain RCH1

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