Task 3. Microbiological Transformations and Mechanisms

Scientific Challenges and Knowledge Gaps

1. Information is needed on the biogeochemical controls on mercury methylation as MeHg is not correlated to the dissolved and total Hg in water at UEFPC.
2. Geochemical controls and dominant microbial species responsible for methylation or demethylation not identified and biochemical pathways, enzymatic mechanisms, and genetic controls for Hg methylation are poorly understood
3. Microbial oxidation of U(IV) and U0 are not well understood

Objectives

1. Increase understanding of the microbial processes that contribute to observed concentrations of MeHg in the ORR system, thus addressing Knowledge Gaps 1 and 2 (above)
2. Understand the importance of biological oxidation on the stability of U in the subsurface thus addressing Knowledge Gap 3.

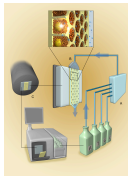
Primary Hypothesis

H1 Hg speciation and transformation are coupled with redox reactions of aqueous species (e.g., Fe, DO, DOM, S) and influenced by solution pH, Eh, ionic strength and composition.

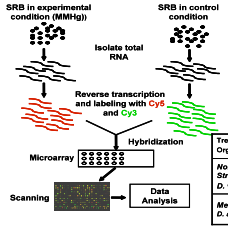
H2 Rates of microbial methylation are influenced by available Hg species that constitute only a small fraction of total aqueous Hg in water.

H4 SRB are the dominant microbes responsible for Hg methylation, but other groups of microbes also contribute directly or indirectly to net MeHg production [e.g., by generating Fe(II)].

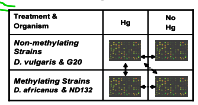
Gene Expression Approach



Sequencing by synthesis on a 454 instrument



- Use whole genome microarrays to compare gene expression in *methylating and non-methylating strains* in response to Hg
- Whole genome arrays will also be used to compare gene expression in *non-methylating strains* with and without Hg



Tasks

3.1 Elucidate the genetic and molecular mechanisms of Hg methylation to help assess environmental controls in the field using comparative gene expression, mutagenesis, and complementation

3.2 Examine the geochemical influences on Hg methylation and gene expression in laboratory studies complementing studies in tasks 1 and 2.

3.3 Produce a better set of community assessment tools more directly tied to Hg methylation and demethylation, in order to refine understanding of the relationships among community structure, net methylation rates, and geochemical conditions

3.4 Test iron oxidizing bacteria for the ability to oxidize U and the conditions affecting the rates of oxidation

Tasks 3.1 and 3.2 are a collaboration with Judy Wall and Cindy Gilmore

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Task 3.2 will start this summer and we have been sampling field sites in Oak Ridge to establish the range of geochemical conditions to be used in the experiments with the Hg methylating strains

In Task 3.3 task we will develop a new version of the FGA using information from the literature for deployment at the field site (Task 1.3)

Preliminary studies underway using the existing version of the FGA at a control site and several contaminated site (see related poster)

In Task 3. 4 we are using *Acidithiobacillus ferrooxidans* as model organism to study genetic responses

We have obtained cultures of Acidithiobacillus ferrooxidans and are testing pH tolerance in laboratory media and have found that it tolerates and grows in near neutral (pH 6) media

We are designing the whole genome microarray and will print and test it this FY

Current Activities and Results

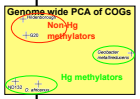
Our primary activates to date have been on Task 1 (see related poster)

We have obtained draft sequences of two methylating strains and identified candidate genes using comparative genomics approaches to identify candidate genes and have designed expression arrays

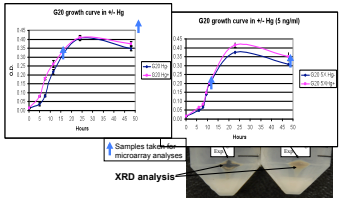
Several of the candidate genes have been cloned from the methylating Desulfovibrio into the non-methylating

We are conducting gene expression studies in the non-methylating strains in the presence and absence of Hg

	Genome Name	
	D. africanus	*D. desulfuricans* ND132
Scaffolds	1,199	676
% G+C	61	65
Bases	4,148,643	3,825,878
Coding bases	3,542,129	3,409,160
Genes	4,777	4,116
CDS	4,679	4,012
CDS %	97.95%	97.47%
w/ Func Pred	3147	2840
Enzymes	784	863
KEGG	651	698
Not In KEGG	4028	3314
COG	3038	2808
Pfam	2997	2808
TIGRfam	954	967



D. desulfuricans G20 Growth in +/- Hg



Gene	Predicted function	Top BLASTP Score (CDS)[1]					
		Daf	ND132	G20	DvH	Gmet	Gsul
2500122319	COG2226: SAM-dependent methyltransferases	2.e-06 (2500128714)	X[2]	NH[3]	NH	2.e-12 (Gmet_2177)	6.e-06 (GSU3022)
2500126448	COG2230: Cyclopropane fatty acid synthase/related methyltransferases	X	6.e-21 (2500123538)	NH	NH	6.e-50 (Gmet_1733)	1.e-50 (GSU1214)
2500123538	COG4122: Predicted O-methyltransferase	3.e-15 (2500126448)	X	NH	NH	6.e-15 (Gmet_1733)	2.e-14 (GSU1214)

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