

**Mercury Biogeochemistry in Great Salt Lake:
The Role of Microorganisms in Methylation**

Funded by The Utah Department of Natural Resources, Forestry, Fire and State Lands.

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I. Proposal Abstract:

Mercury (Hg) contamination of Great Salt Lake is of heightened concern due to the amount of methylated Hg present in the deep brine layers and sediment. This methylation is indeed a biological process, conducted by microorganisms in the ecosystem, and the effect can be amplified up the food chain. Our goal was to identify bacteria and archaea that are capable of this activity by surveying genes for such pathways and correlating this with cultivation data and methylation potential. By identifying the starting point of methylHg formation in the ecosystem, we can then strategize intervention. The ultimate goal was to provide the baseline data needed to design bioremediation systems.

II. Progress:

We are pleased to report that we had a successful collaboration with U.S. Geological Survey Naftz and Marvin-DiPasquale (also funded by FFSL), and we were able to link efforts with respect to sampling and data sharing. This model of funded groups working together in Utah on a project that is critical to the local and regional economy continues to be refined and has been leveraged to generate significantly more data with no addition in cost.

Sampling Sites. Following funding of last year's proposal, we began a collaboration with Naftz and DiPasquale, and this resulted in several minor changes to our proposed sampling scheme. In joining efforts, we were able to have access to more samples than originally planned and focused our resources on collecting the datasets previously proposed, while obtaining access to additional chemical and physical measurements obtained by USGS collaborators. The sites sampled are listed in Table 1.

Cultivation. Brine samples were cultivated with and without enrichment in liquid growth media (e.g. Baxter *et al.*, 2007) and then transferred to solid media plates, all under anaerobic conditions.

Based on physiological properties, specific colonies were selected for genetic and biochemical screening for Hg transformation activities. Since microorganisms growing in high levels of Hg contamination are likely to have developed mechanisms to resist its toxic effects, we tested isolated microbes from water and sediment samples on growth media that was supplemented with 25 to 400 ppm Hg. Table 1 shows media plates which had growth of microorganisms correlated with percent salinity and

location of original brine/sediment sample. Colonies from the plates with successful growth (Table 1) were isolated on individual plates. Currently, these 14 individual species of microorganisms are being cultivated both under anoxic and oxic cultures in order to prepare enough DNA for sequence analysis and taxonomic identification. One of these strains is growing on 400 ppm HgCl₂, and we are titrating HgCl₂ levels to titrate its Hg tolerance.

Sample Location	Site Type	Salinity (ppt)	pH	12% NaCl	18% NaCl	23% NaCl
				Isolated Recovered+/-		
BR - Pond 5C	freshwater marsh	0.5	7.17	+	-	-
BR - Pond 3E	freshwater marsh	9.0	7.56	+	-	-
Farmington Bay	Saltmarsh	53.0	8.31	+	-	-
Bear River Bay	Saltmarsh	50.7	7.48	+	-	-
South Shore	Brackish open water	19.6	7.91	+	-	+
Ogden Bay	Brackish open water	31.5	7.3	+	+	+
North Basin	Hypersaline	130	7.14	-	+	-
Gunnison Island	Hypersaline	293	6.96	-	+	+

Table 1. Anaerobic Cultivation Results. Liquid minimal growth media (containing 12%, 18% and 23% NaCl) was inoculated with 100 µl of each field sample.

Aerobic Cultivation. The number of Hg resistant heterotrophic aerobic microbes was obtained to assess the potential of GSL sediment microbial communities to withstand the toxicity of Hg. In general, 1-10% of culturable aerobic heterotrophs in soils and sediments are resistant to Hg (Barkay, 1992). Therefore, the media Hg concentration at which approximately 90-99% of these organisms were inhibited was determined for three samples (one representative from each type of saline environment). To do this, serial dilutions of sediment samples were prepared and 100 µL was spread-plate inoculated on solid modified growth medium (MGM) with appropriate salinity containing various concentrations of HgCl₂. Plates were incubated at 28 °C until sufficient growth was seen to make this determination.

Sample Location	Site Type	Porewater Salinity (ppt)	salinity of media (%)	incubation period (days)	estimated cfus/g of sediment	Hg concentration inhibiting approximately 90-99% of cfus (μM)
Farmington Bay	Saltmarsh	31	3	7	4.9×10^7	50
Ogden Bay	Brackish open water	75	7.5	7	3.2×10^8	50
Gunnison Island	Hypersaline	304	18, 23	12, 24	1.3×10^8 (for both salinities)	10

Table 2. Aerobic Cultivation Results. Solid minimal growth media was inoculated with 100 μl of serially diluted sediment samples.

Several different colony morphologies from the Gunnison Island platings were described. Representative colonies growing on Hg-containing plates were chosen for isolation and further study. Selected colonies were purified by the quadrant streak plating (QSP) method on 18 or 23% salinity MGM (depending on salinity of original dilution plate) amended with 10 μM HgCl_2 (Table 2), followed by incubation at 28 °C. Once isolated colonies were observed, QSP was repeated two more times to ensure purity. Isolated colonies were then used to inoculate MGM broth (18 or 23% salinity, 10 μM HgCl_2) and incubated at 28 °C while shaking until cultures reached turbidity. Subsamples of these cultures were used for (i) genomic DNA isolation and (ii) glycerol stock preparation. We currently have 7 isolates preserved for future studies regarding their mercury resistance.

Pyrosequencing. We have generated ~ 3000 16S rRNA gene sequences specific for archaeal and bacterial microorganisms in each of the eight sediment samples in Table 1 (See Fig 1B), or ~48,000 rRNA gene sequences in total. This dataset affords an unprecedented view of the microbial biodiversity of GSL and has served a critical role in identifying potential differences in the microbial communities associated with sediments that exhibit differences in Hg methylation potentials that we and our USGS collaborators have measured. Important differences are already apparent in the datasets, such as the relative abundance of sulfate- and iron-reducing taxa among the different sites, and the relative biodiversity (number of species) at each site, which is in the process of being modeled as a function of geochemical and activity data. We reallocated funds earmarked for sampling efforts to additional sequencing efforts, and obtained RNA-based sequence from these eight sediment sites. RNA was extracted from each of the eight sediments, reverse-transcribed to obtain total cDNA, and subjected to quantitative amplification (See Fig. 1A) to better understand the abundance of active archaeal and bacterial populations in the sediment environments.

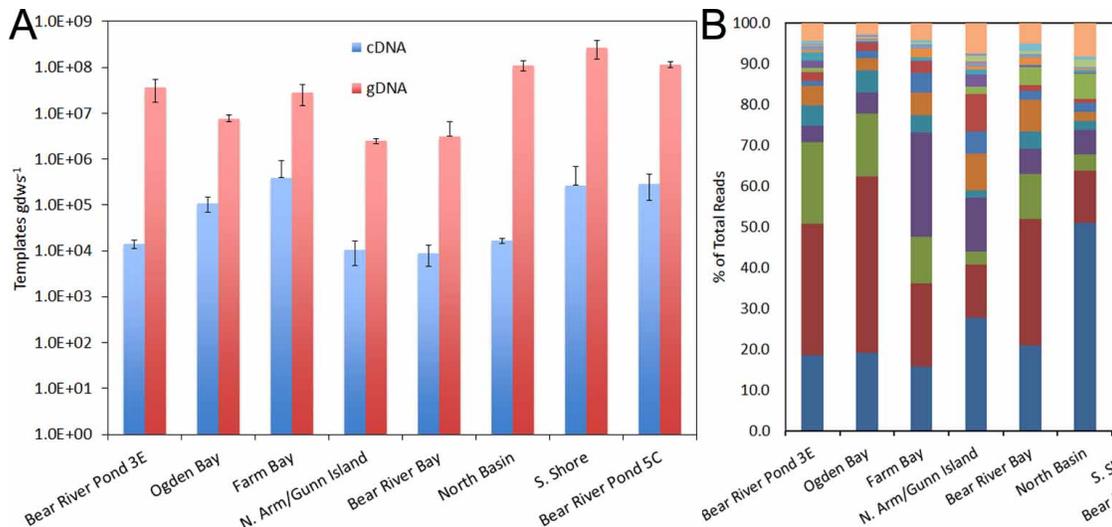


Fig. 1. Panel A, the abundance of bacterial 16S SSU rRNA genes in the RNA fraction (cDNA) and the DNA fraction (gDNA). Panel B, the relative abundance of bacterial 16S rDNA genes associated with sediments sampled from the eight sites. Analogous Data is available for archaeal SSU rRNA genes and their

We are currently awaiting (projected data delivery date of 07/06/2012) the 16S cDNA pyrosequencing results, which should yield ~ 3000 reads per archaeal and bacterial assemblage, or an additional ~48,000 reads. This will afford a number of unique insights into the ecology of these eight sediment environments, including a calculation of the active versus inactive fraction of each community. Since this cDNA sequence data is a reflection of the active assemblage in each environment, we also expect to observe much stronger links with measured activity potentials, in addition to physical and chemical parameters that we and our USGS colleagues have measured. This will serve to better delineate the populations responsible for the observed activities. To our knowledge, this is the first successful attempt at characterizing the biodiversity of GSL using RNA-based approaches. Importantly, the existing cDNA that was obtained in this round of funding is well-suited and preserved (ammonium acetate/ethanol precipitation and -80°C storage) for additional functional transcript (*dsrAB* as a proxy for sulfate-reducing taxa, *merA* for Hg detoxifying taxa, etc.) profiling that could also be performed in future investigations.

Methylation Activities. Based on results showing a clear distinction in methylation potentials (DiPasquale), community composition (Boyd, see Fig. 1B), and the relative abundance of lineages putatively involved in sulfate reduction across the eight sediment sites, the number of sulfate reducing bacteria was estimated by the most probable number (MPN) approach focusing on complete oxidizer SRB, those that can oxidize acetate as a sole carbon and energy source, and on total SRB, those that oxidize a variety of compounds mostly by converting them to acetate. Clear distinctions between the salt tolerance of SRB (Oren, 2006) and the pathways for Hg methylation by SRB (Ekstrom et al., 2003; Ekstrom and Morel, 2008) suggest that the distribution of methylation activities in GSL may be spatially constrained by salinity. Results (Table 2) showed that the number of SRB was much higher in low salinity

sediment of Pond 3E (pore water salinity 1.5%) and the intermediate salinity sediment Bear River Bay (5.6%) than in the high salinity sediment from North Basin (22.8%). In addition, the proportion of complete oxidizer SRB increased with salinity with North Basin sediment containing only complete oxidizer SRB.

Sediment	% Salinity	Substrate	estimated # SRB, cells/g sed	Proportion of complete oxidizers
Bear River Pond 3E	1.5	Acetate Only	3.5×10^3	2.4×10^{-4}
Bear River Pond 3E	1.5	Five Substrates	1.1×10^7	
Bear River Bay	5.6	Acetate Only	5.4×10^4	1.2×10^{-2}
Bear River Bay	5.6	Five Substrates	4.6×10^6	
North Basin	19	Acetate Only	5.4×10^2	0
North Basin	19	Five Substrates	0	

Table 2. MPN estimates of complete and incomplete oxidier SRB in 3 sediment samples. Calculated # organisms based on MPN table. *No positive growth has been observed to date.

These results may explain the observed low methylation potentials in high salinity sediments (<0.1 pg MeHg /g sed/day for North Basin) relative to the brackish Bear River Bay (19.5+1.9 pg MeHg/g sed/day) and the freshwater marsh Pond 3E (41.6+4.7 pg MeHg /g sed/day). (All methylation potentials were obtained by DiPasquale.)

In order to examine how the patterns in SRB populations are related to MeHg accumulation, we determined potential methylation rates in these sediments and how they are affected by the addition of metabolic stimulators and inhibitors (Fig. 2). Results clearly show that the highest potential methylation rates occurred in the freshwater marsh pond and that this potential was very low in the hypersaline site. In both the freshwater and brackish sediments, activities were stimulated by the addition of sulfate and ferric iron and inhibited by molybdate, clearly implicating both sulfate and iron reducers in Hg methylation in GSL sediments (Fig. 2).

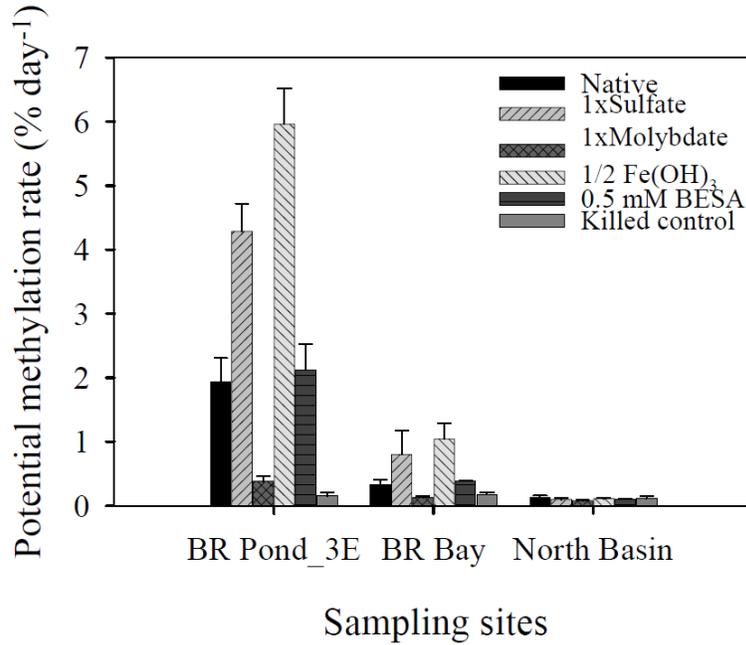


Fig. 2: Effects of the additions of stimulators and inhibitors on potential methylation rates in three select sediment samples

III. Products:

"Populations of Sulfate Reducing Bacteria and Potential Hg Methylation in Response to a Sediment Salinity Gradient in Great Salt Lake, Utah." Rainier Pineda, Ri-Qing Yu, Mark Marvin Di-Pasquale, and Tamar Barkay. North Eastern Microbiologists: Physiology, Ecology and Taxonomy, 2012. Blue Mountain Lake, NY, June 29-30, 2012

"Microbial Isolations from Mercury-Contaminated Regions of Great Salt Lake." Austin Wood, Thomas Stevens, Caleb West, Jaimi Butler, and Bonnie K. Baxter. Undergraduate Research Fair, Westminster College, Salt Lake City, UT.

IV. References:

Barkay T. (1992) Mercury Cycle. Encyclopedia of Microbiology, Vol. 3, pp. 65–74. Academic Press.

- Baxter B.K., Eddington B., Riddle M.R., Webster T.N. and Avery B.J. Great Salt Lake Halophilic Microorganisms as Models for Astrobiology: Evidence for Desiccation Tolerance and Ultraviolet Radiation Resistance. In: Hoover R.B., Levin G.V., Rozanov A.Y., and Davies P. C.W. (eds.) *Instruments, Methods, and Missions for Astrobiology X*, 6694:669415. SPIE, Bellingham, WA, 2007.
- Ekstrom EB, Morel FMM. (2008) Cobalt limitation of growth and mercury methylation in sulfatereducing bacteria. *Environ Sci Technol* 42:93-99.
- Ekstrom EB, Morel FMM, Benoit JM. (2003) Mercury methylation independent of the acetyl-coenzyme a pathway in sulfate-reducing bacteria. *Appl. Environ. Microbiol* 69:5414-5422.
- Oren, A. (2006) Life at high salt concentrations. *Prokaryotes* 2:263-282