Mercury Biogeochemistry in Great Salt Lake: Delineating the Microorganisms involved in Methylation and Ecosystem Detoxification

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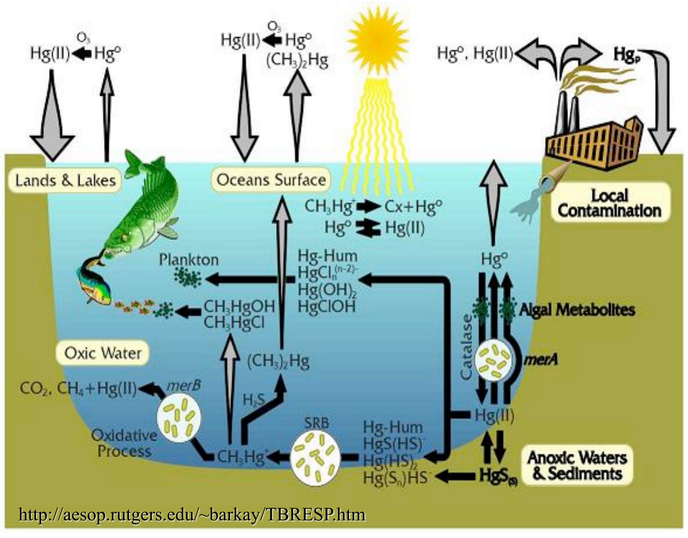
Introduction

Microorganisms within the deep brine and sediment layers at Great Salt Lake are suspect to be responsible for the process of methylating mercury (Hg). Methylmercury (MeHg) is an organometallic cation which has a greater capability of incorporating itself into biological tissues. The intoxicating effects of the methylated mercury tend to increase as it accumulates higher within the ecosystem. High levels of Hg contamination at GSL, which lead to an increase in the microbial production of MeHg, have become the main concern of this project. Our objective is to identify the species of bacteria and archaea that are specifically responsible for this process, and to better understand the biochemical pathways utilized by these microorganisms to convert Hg to MeHg.

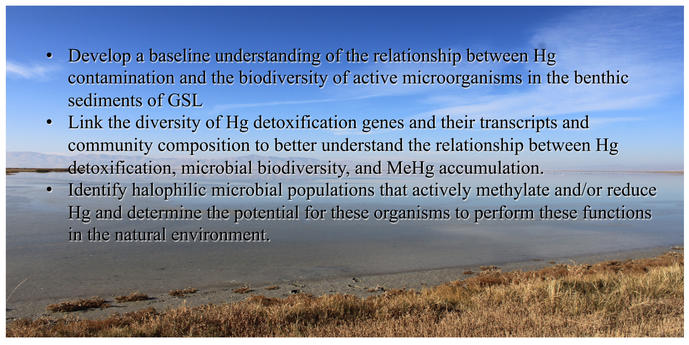
The potential for MeHg production in the isolated microbes will be tested by selectively growing the isolates in the presence of Hg. The microorganisms that have the ability to withstand Hg's toxic effects are likely to possess the machinery required for the production of MeHg. Once isolated and tested for resistance to Hg, the microbes will be tested to detect for the transcription of any genes involved in the *mer* systems, which are the enzymatic machinery that would allow for them to withstand the presence of Hg and allow it to produce MeHg (for example, the mercuric reductase enzyme (*merA*), and the organoHglyase (*merB*).

Once the species of microorganisms that have the potential for transforming Hg contamination have been identified, and once the biological systems that these microbes are utilizing are better understood, strategies for intervention and bioremediation can then be developed after obtaining this information.



http://aesop.rutgers.edu/~barkay/TBRESP.htm

Objectives



- Develop a baseline understanding of the relationship between Hg contamination and the biodiversity of active microorganisms in the benthic sediments of GSL
- Link the diversity of Hg detoxification genes and their transcripts and community composition to better understand the relationship between Hg detoxification, microbial biodiversity, and MeHg accumulation.
- Identify halophilic microbial populations that actively methylate and/or reduce Hg and determine the potential for these organisms to perform these functions in the natural environment.

Methods

1. Cultivation & Isolation

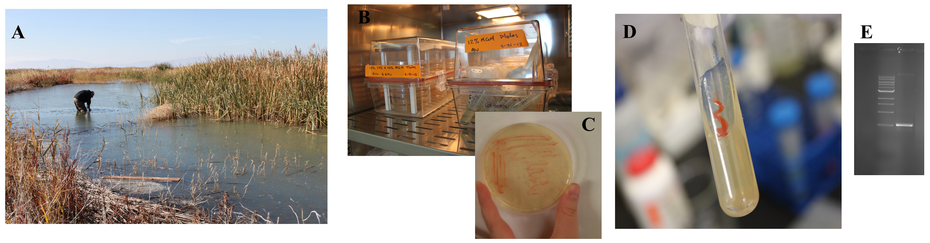
Brine and sediment samples taken from eight different locations, were used to inoculate liquid broth cultures with media of various salt concentrations (12%, 18% and 23% MGM). To simulate conditions similar to that of the benthic regions of the lake the isolates were grown under anaerobic conditions. Streaking inoculums onto solid media allows for isolation of single colonies.

2. Genetic and biochemical screening for Hg transformation activities

Once isolated into single colonies, the microbes are cultured on growth media that contains 25-400ppm HgCl. Titrating the amount of HgCl to supplement the growth media will help determine how resistant the microbes are to Hg's toxic effects.

3. Identification

After performing DNA extractions on the isolates, amplification of the DNA sequence that encodes for the 16S rRNA by PCR is carried out so that this region of the microorganism's DNA can be sequenced. After obtaining sequencing data, the sample is identified using a BLAST (Basic Local Alignment Search Tool) program.



A. Collecting brine samples in the field. **B.** Anaerobic growth chambers. **C.** Streaked agar/MGM plate. **D.** Halorubrum lacusprofundi subspecies or new species. **E.** Successful PCR product of 16S rRNA.

Results

TABLE 1

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. Brine samples were used to inoculate liquid Modified Growth Medium (MGM) of varying salinities. The liquid cultures were then streaked onto solid media with respective salinity.

Figure 1. Diversity of microbial population based on depth and salinity. Represented here in this histogram is the relative abundance of bacterial orders in a water column (Meuser *et al.,* submitted)

Figure 2. Pie chart comparing the bacterial diversity in upper and lower brine layers of the South Arm of Great Salt Lake.

Figure 1.

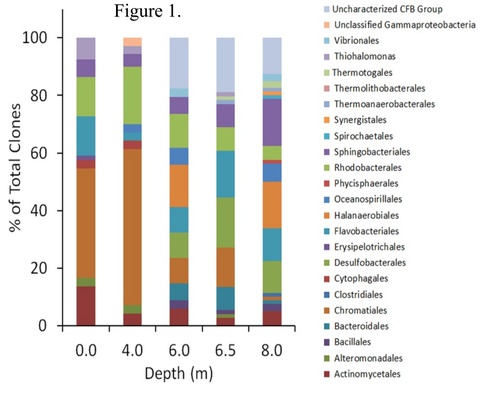
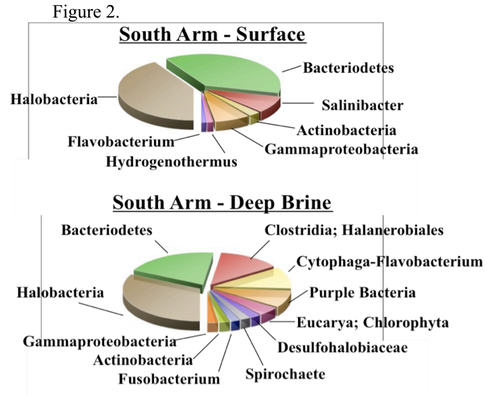
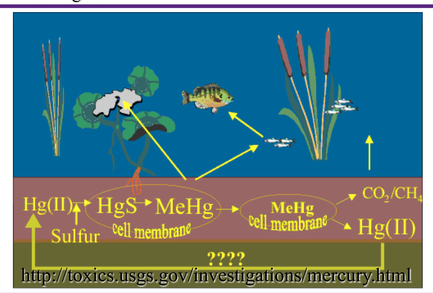


Figure 2.



Discussion

- While microbes play a central role in the methylation process of Hg, there are some microbes that have the ability to degrade MeHg into its elemental form which is less hazardous. Something to think about when developing potential bioremediation strategies.



http://toxics.usgs.gov/investigations/mercury.html

Conclusion

Currently there are several different strains of anaerobic bacteria (see Table 1. for site specifics) and are in the process of isolating them into pure cultures. The anaerobic strains will be tested for resistance to Hg.

A strain of aerobic Hg resistant halophile was sent to our lab from Dr. Carol Litchfield's which has recently been identified based on 16S rRNA sequence.

Future Work/Collaborations

Dr. David Naftz at the Salt Lake City USGS office is a hydrogeologist who had studied the Hg contamination of GSL. We will piggy-back our data analysis efforts with Dr. Naftz such that we can coordinate data from microbes in our samples to his Hg analysis. We will also share data freely between the projects.

Drs. Frank Black and Christine Stracey at Westminster College are submitting a FFSL proposal to look at the movement of the Me-Hg from the microbial realm into the brine fly and to the lakeshore terrestrial ecosystem through arachnids. We work hand-in-hand with this research group.

Acknowledgements

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