Genetic Origins of Mercury Resistance in Great Salt Lake Microorganisms

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Introduction

Extremophiles are a diverse group of organisms, typically Bacteria and Archaea, that can inhabit extreme environments, such as geysers, deserts, and saline lakes. Their abilities to withstand extremely dry, hot, salinic, acidic, and mercuric conditions have made these microorganisms admirable astrobiological models for life on other planets[1].

Background

Methylmercury (CH_3Hg) is a neurotoxin that accumulates in aquatic environments due to the actions of microorganisms, which can produce this biologically relevant organic form from elemental mercury (Hg) (Figure 1, right).

Many species of microorganisms have shown resistance to Hg and can thrive in polluted waters. Recent studies have shown that Hg resistance in Bacteria and Archaea arises from one of two gene pairs, *mer*AB or *hgc*AB[3,4]. The *mer*AB system produces gene products that allow the organism to convert CH_3Hg into elemental Hg[3]. Conversely, the *hgc*AB system coverts Hg into CH_3Hg[4] (Table 1). Through these mechanisms, microorganisms can play a significant role manipulating the health of aquatic ecosystems.

Mercury Resistant Genotypes

Gene	*Function*
***hgc*A**	Aids in methylation of Hg, gene products currently unknown[4]
***hgc*B**	Aids in methylation of Hg, gene products currently unknown[4]
***mer*A**	Aids in demethylation of CH_3Hg through production of mercury reductase[3]
***mer*B**	Aids in demethylation of CH_3Hg through production of organomercurial lyase[3]

Table 1. Each mercury-resistant genotype consists of two gene pairs. The hgcAB gene pair assists in mercury resistance by methylating Hg[4]. The metabolic pathway in which this occurs is currently being studied and specific gene products have yet to be published. The merAB system, on the other hand, is more defined. The gene products of this system, mercury reductase and organomercurial lyase, work together to demethylate CH_3Hg[3].

Due to natural and industrial influences, the Great Salt Lake (GSL) has accumulated Hg within its waters. Although the lake has no fish, mercuric bioaccumulation has extended from the microbial and shrimp populations to terrestrial animals, such as spiders and birds. As previously suggested, the GSL microbes may have a significant influence over the production of CH_3Hg from Hg inputs. Therefore, defining the genotype of mercury-resistant GSL microorganisms is essential to understanding the behavior of CH_3Hg in this ecosystem and may inform future bioremediation attempts on the lake.

Hypothesis

GSL microorganisms that demonstrate a robust resistance to mercuric conditions will express either the *hgc*AB or *mer*AB genotype.

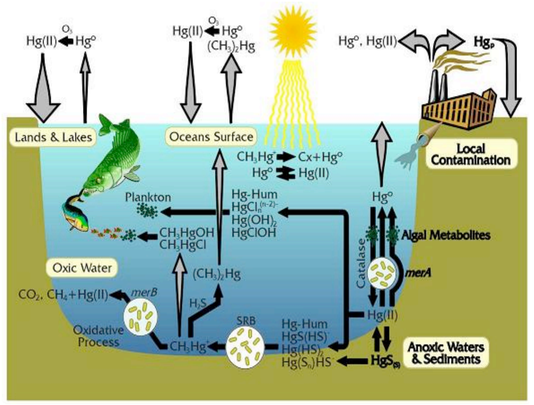

Figure 1. Through the actions of microorganisms and local industry, Hg and CH_3Hg concentrations are manipulated and bioaccumulated in aquatic ecosystems[2].

Objectives

1. Identify mercury-resistant microorganisms in the GSL.
2. Establish the range of mercury resistance.
3. Determine the mercury-resistant genotype.

Materials & Methods

Overview: Microorganisms will be harvested from the GSL and cultivated on increasing concentrations of mercury. The most resistant microbes will undergo genetic analysis to identify resistant genotypes in the population.

Harvest, Cultivation, & Isolation

Anaerobic halophiles, "salt-loving" microorganisms, were obtained from the deep brine layer of the GSL. This method was repeated in 8 different locations of the lake in order to observe a broader sample of the GSL microbial community. Locations are listed in Tables 2, 3 & 4.

Samples were cultivated in broth culture before being transferred to petri dishes containing modified growth medium (MGM)[5] infused with 5 ppm mercury chloride ($Hg(II)Cl_2$), a form of Hg represented in the GSL. The plates were representative of a range of salinities, 12%, 18%, 23%, and 25%, as well. Cultures were incubated at 37° C in anaerobic chambers.

Determining Mercury Resistance

Individual colonies were selected from the 5 ppm $Hg(II)Cl_2$ plates and transferred to 10 ppm $Hg(II)Cl_2$ plates with corresponding salinities. After sufficient colony growth was observed, the specimens were transferred to 20 ppm $Hg(II)Cl_2$. In this way, mercury concentrations were slowly increased in order isolate the most resistant microorganisms. All plates were incubated at 37° C in anaerobic chambers.

Genetic Analysis

The microorganisms that demonstrated the greatest resistance to $Hg(II)Cl_2$ are being analyzed for *hgc*AB and *mer*AB. Additionally, the 16S rRNA gene, which will determine the identity of these microbes, will be assessed, as well. Analysis of DNA extracts will be carried out with PCR amplification, gel electrophoresis, and genetic sequencing.

Results

Mercury-resistant halophiles have successfully been cultivated on 5, 10, and 20 ppm $Hg(II)Cl_2$ plates across various salinities, distributions represented Tables 2, 3, & 4. Of the eight sampling sites, 46, 26, and 25 distinct colonies were exhibited on 5, 10 and 20 ppm $Hg(II)Cl_2$ plates, respectively.

Isolates on 5 ppm $Hg(II)Cl_2$ MGM

Site	*12%*	*18%*	*23%*	*25%*
Bear River Bay	1	1	2	1
Bear River Pond 3E	3	1	1	1
Bear River Pond 5C	1	1	0	0
South Shore	1	1	1	1
North Basin	3	3	2	1
Farmington Bay	2	1	1	1
North Arm/Gunnison Island	2	2	2	2
Ogden Bay	5	1	0	1

Table 2. From the 8 sampling locations, 46 distinct colonies were presented on plates containing 5 ppm $Hg(II)Cl_2$.

Isolates on 10 ppm $Hg(II)Cl_2$ MGM

Site	12%	18%	23%	25%
Bear River Bay	0	1	1	1
Bear River Pond 3E	2	1	0	1
Bear River Pond 5C	0	1	0	0
South Shore	1	0	0	1
North Basin	3	0	0	1
Farmington Bay	1	1	0	1
North Arm/Gunnison Island	2	1	1	2
Ogden Bay	2	0	0	1

Table 3. Of the 46 colonies, only 26 colonies continued to grow on 10 ppm $Hg(II)Cl_2$ plates.

Isolates on 20 ppm $Hg(II)Cl_2$ MGM

Site	12%	18%	23%	25%
Bear River Bay	0	1	1	1
Bear River Pond 3E	2	1	0	1
Bear River Pond 5C	0	1	0	0
South Shore	1	0	0	1
North Basin	3	0	0	1
Farmington Bay	1	1	0	1
North Arm/Gunnison Island	2	1	1	1
Ogden Bay	2	0	0	1

Table 4. Of the 26 colonies grown in 10 ppm $Hg(II)Cl_2$ all but one colony thrived on the 20 ppm $Hg(II)Cl_2$ plates.

Results Continued

Four 20 ppm $Hg(II)Cl_2$ isolates were analyzed for *hgc*A, *mer*AB, and 16S rRNA. Gel electrophoresis and sequencing of the resulting PCR products did not yield conclusive results. According to the data shown in Figures 2 and 3, two strains demonstrated both genotypes. However, gene sequencing was not successful and could not confirm these findings. More tests will be needed before drawing any conclusions.

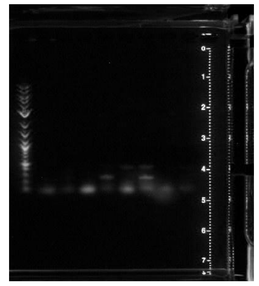

Figure 2. From left to right, *hgc*A and B PCR product alternates after the DNA ladder shown in lane 1. Lanes 2 & 3 and 4 & 5 correspond with the organisms represented in lanes 2 & 3 and 4 & 5 of Figure 3, respectively.

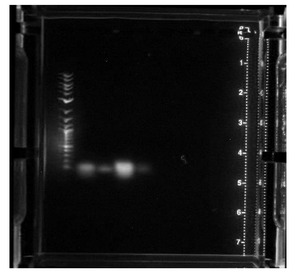

Figure 3. From left to right, *mer*A and B PCR product alternates after the DNA ladder shown in lane 1. Lanes 2 & 3 and 4 & 5 correspond with the organisms represent in lanes 2 & 3 and 4 & 5 of Figure 2, respectively.

Conclusions

GSL microorganisms demonstrate a robust resistance to mercury chloride, as was shown in Tables 2, 3, and 4. The mercury-resistant genotype of these halophiles, however, were not identified. Initial analysis of four 20 ppm $Hg(II)Cl_2$ isolates suggests that two microbes express the *hgc*AB genotype while two others seem to demonstrate both *hgc* and *mer* genotypes (Figures 2 & 3). Despite these results, genetic sequencing was not conclusive. More experiments will be needed before extending any conclusions.

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