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.

Background

Methymercury (CH$_3$Hg) 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, merA or hgcAB. The merA system produces gene products that allow the organism to convert CH$_3$Hg into elemental Hg. Conversely, the hgcAB system converts Hg into CH$_3$Hg (Table 1). Through these mechanisms, microorganisms can play a significant role in managing the health of aquatic ecosystems.

Table 1. Each mercury-resistant genotype consists of two gene pairs. The hgcAB gene pair assists in mercury resistance by methylation of Hg, whereas the merA system converts Hg into elemental Hg. The metabolic pathway in which this occurs is currently being studied and specific gene products have yet to be published. The merA system, on the other hand, is more defined. The gene products of this system, mercury reductase and organomercurial lyase, work together to demethylate CH$_3$Hg.

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 poplar shrimp to terrestrial animals, such as spiders and birds. As previously suggested, the GSL microbes may have a significant influence over the production of CH$_3$Hg from Hg inputs. Therefore, defining the genotype of mercury-resistant GSL microorganisms is essential to understanding the behavior of CH$_3$Hg in this ecosystem and may inform future bioremediation attempts on the lake.

Hypothesis

GSL microorganisms that demonstrate a robust resistance to mercury compounds will express either the hgcAB or merAB genotype.

Materials and 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) infused with 5 ppm mercury chloride (HgCl$_2$) as 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 HgCl$_2$ plates and transferred to 10 ppm HgCl$_2$ plates with corresponding salinities. After sufficient colony growth was observed, the specimens were transferred to 20 ppm HgCl$_2$. 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 HgCl$_2$ are being analyzed for hgcAB and merAB. 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

Table 1. Each mercury-resistant genotype consists of two gene pairs. The hgcAB gene pair assists in mercury resistance by methylation of Hg, whereas the merA system converts Hg into elemental Hg. The metabolic pathway in which this occurs is currently being studied and specific gene products have yet to be published. The merA system, on the other hand, is more defined. The gene products of this system, mercury reductase and organomercurial lyase, work together to demethylate CH$_3$Hg.

Table 2. From the 8 sampling locations, 4 distinct colonies were presented on plates containing 5 ppm HgCl$_2$.

Table 3. Of the 46 colonies, only 26 colonies continued to grow on 10 ppm Hg(II)Cl$_2$ plates.

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 hgcAB, merAB, 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.

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 hgcAB 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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References


