Microbial pathways for the reduction of mercury in saturated subsurface sediments

Final report

Submitted by:

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Mercury is a component of mixed wastes that have contaminated vast areas of the deep subsurface as a result of nuclear weapon and energy production. While this mercury is mostly bound to soil constituents episodes of groundwater contamination are known in some cases resulting in potable water super saturated with Hg(0). Microbial processes that reduce Hg(II) to the elemental form Hg(0) in the saturated subsurface sediments may contribute to this problem. When we started the project, only one microbial pathway for the reduction of Hg(II), the one mediated by the *mer* operon in mercury resistant bacteria was known. As we had previously demonstrated that the *mer* mediated process occurred in highly contaminated environments (Schaefer *et al.*, 2004), and mercury concentrations in the subsurface were reported to be low (Krabbenhoft and Babiarz, 1992), we hypothesized that other microbial processes might be active in reducing Hg(II) to Hg(0) in saturated subsurface environments. The specific goals of our projects were:

1. Investigating the potential for Hg(II) reduction under varying electron accepting conditions in subsurface sediments and relating these potential to *mer* gene distribution.
2. Examining the physiological and biochemical characteristics of the interactions of anaerobic bacteria with mercury.

The results are briefly summarized below with references to published papers and manuscripts in preparation where details about our research can be found. Additional information may be found in copies of our published manuscripts and conference proceedings, and our yearly reports that were submitted through the RIMS system.

Hg(II) reduction under denitrifying and iron reducing conditions: We initially evaluated the toxicity of mercury to denitrifying Oak Ridge FRC sediment enrichments. We derived a nitrate reducing enrichment culture by incubating sediment from the background area in an artificial ground water medium (AGW) supplemented with 10 mM acetate and 5 mM nitrate. When nitrate reduction was established, we used this enrichment to inoculate serum bottles containing AGW, 10% (weight:volume) autoclaved FRC sediment, 10 mM acetate, 5 mM nitrate, and increasing concentrations of mercury, provided as HgCl₂ (53 nM to 24 μ M). Nitrate reduction was monitored for up to 220 days and the time it has taken for the enrichment to initiate nitrate reduction was used as an indication of mercury toxicity. Unexposed microcosms reduced the added nitrate in a period of 13 days. An inverse relationship was observed between Hg concentrations and onset of denitrification: nitrate reduction occurred on the 13th day of incubation with no addition of HgCl₂, but occurred on the 55th day with 312 nM of HgCl₂. Above 1 μ M of

HgCl₂, nitrate reduction was not detected even on the 220th day. These findings are being prepared for publication (Wiatrowski et al., in preparation).

Increasing Hg concentrations also decrease the number of dominating species selected in the enrichments as revealed by tRFLP patterns. The enrichment that was supplemented with 312 nM of HgCl₂, had a single tRFLP peak was most closely related to that of the alphaproteobacterium *Bradyrhizobium* sp., a known denitrifier, and a PCR fragment amplified from this enrichment by *merA*-specific primers, had 89% inferred sequence identity to MerA in *Acinetobacter* sp. suggesting the selection of Hg resistant bradyrhizobia in denitrifying enrichments. The role for this genus in the response to Hg in the FRC subsurface sediments was supported when Hg resistant denitrifying isolates from the enrichments were related to bradyrhizobia, one of which had a *merA* with 67% inferred sequence identity to MerA of the actinobacterium *Nocardioides* sp. Thus, denitrifying bradyrhizobia might be major players in Hg detoxification in the subsurface. These findings are being prepared for publication (Wang et al., in preparation). **Together, these results suggest that (i) denitrification in subsurface sediments is highly sensitive to inhibition by Hg; (ii) denitrification in the enrichments was restored upon selection and growth of Hg resistant bacteria; (iii) horizontally transferred *merA* genes might have contributed to this enrichment.** While initial experiments with iron reducing enrichments did not provide reproducible results, later incubations were successful and the analyses and further examination of these incubation is currently progressing.

The reduction of Hg(II) by metal reducing anaerobic bacteria: One of the major findings of our project is that anaerobic metal reducing bacteria such as *Shewanella oneidensis* MR-1 and *Geobacter* spp. reduce Hg(II) to Hg(0) by a novel pathway that is not related to the *mer*-mediated resistance (Wiatrowski *et al.*, 2006). These strains are of great interest for bioremediation in the subsurface due to their ability to reduce toxic metals and radionuclides. Additionally, these strains do not display Hg resistance typical of an organism with a *mer* operon. In the presence of 200 nM Hg(II), live cells of strain MR-1 grown aerobically reduced 83±6.3% of the Hg(II), whereas autoclaved cells reduced 21±0.9% in 24 hours. Hg(II) reduction by MR-1 also occurred with iron oxyhydroxide and fumarate as terminal electron acceptors. Reduction of Hg(II) showed a strong dependence on the presence of an electron donor and an electron acceptor, as incubation of cells in media which lacked either resulted in activity that is not significantly different from that of autoclaved cells ($p > 0.01$). Unlike *mer*-mediated Hg(II) reduction, this activity is not inducible, as exposed cells and unexposed cells had a specific activity for reduction of Hg(II) of 3.14 ± 0.25 and 3.07 ± 0.35 nmol min⁻¹ mg protein⁻¹ respectively. Live and autoclaved cells of *G. sulfurreducens* PCA reduced Hg(II) with specific activities of 2.8 ± 1.3 nmol Hg(II) min⁻¹ mg protein⁻¹ and 0.34 ± 0.5 nmol Hg(II) min⁻¹ mg protein⁻¹, respectively. Live and autoclaved cells of strain *G. metallireducens* GS-15 had specific activities of 6.05 ± 1.4 nmol Hg(II) min⁻¹ mg protein⁻¹ and 1.65 ± 0.9 nmol Hg(II) min⁻¹ mg protein⁻¹, respectively. However, Hg(II) reduction is not universal among DMRB or anaerobes, as it was absent in *Anaeromyxobacter dehalogenans* strain 2CP-C, which can reduce iron, and the nitrate reducer *Pseudomonas stutzeri* OX1.

One of the most intriguing finding of these studies was based on the observation when grown using iron oxyhydroxide as a terminal electron acceptors, Hg(II) reduction

only occurred if the strains were grown overnight prior to the addition of Hg(II) and reduction rates were 3 to 4 folds higher relative to rates that were observed using fumarate as an electron acceptor (Wiatrowski *et al.*, 2006). These observations suggested the possibility that Hg(II) was reduced by ferrous iron (Fe(II)) that was the product of ferric iron (Fe(III)) reduction. We therefore investigated the redox transformation of Hg(II) in the presence of the Fe(II)/Fe(III) mixed valence iron oxide mineral magnetite. Kinetic and spectroscopic experiments were performed to elucidate reaction rates and mechanisms. The experimental data demonstrated that reaction of Hg(II) with magnetite results in the loss of Hg(II) and the formation of volatile Hg(0). Kinetic experiments showed that Hg(II) reduction occurred within minutes, with reaction rates increasing with increasing magnetite suspension density (0.05 to 0.2 g/L) and solution pH (4.8 to 6.7), and decreasing with increasing chloride concentration (10^{-6} to 10^{-2} mol/L). Mössbauer spectroscopic analysis of reacted magnetite samples revealed a decrease in Fe(II) content, corresponding the oxidation of Fe(II) to Fe(III) in the magnetite structure. X-ray photoelectron spectroscopy detected the presence of Hg(II) on magnetite surfaces, suggesting that adsorption is involved in the electron transfer process. These results suggest that Hg(II) reaction with solid-phase Fe(II) is a kinetically favorable pathway for Hg(II) reduction in magnetite-bearing environmental systems (Wiatrowski *et al.*, 2009). **Together, we have discovered two new pathways for the reduction of Hg(II) to Hg(0). The first is mediated by metal reducing bacteria, and the second is an abiotic reduction by Fe(II)/Fe(III) bearing minerals.** These processes may play a significant role in the transformation/mobilization of mercury in saturated subsurface sediments where metal reducing bacteria dominate and their activities affect the formation of iron minerals.

References

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- Wiatrowski HW, Ward PM, Young L, Barkay T (2006). Novel reduction of mercury(II) by mercury-sensitive dissimilatory metal reducing bacteria. *Environmental Science and Technology* **40**: 6690-6696.

Wiatrowski HA, John R, Wang Y, Barkay T. Mercury resistance and reduction in denitrifying enrichments of saturated sediments from the the Oak Ridge Field Research Ceneter. In preparation.

Published papers and conference presentations that have resulted of this project

I. Published papers and submitted manuscripts

Ní Chadhain, S., J.K. Schaefer, S. Crane, G.J. Zylstra, and T. Barkay. 2006. Analysis of mercuric reductase (*merA*) gene diversity in an anaerobic mercury-contaminated sediment enrichment. *Environ. Microbiol.* **8**:1746-1752

Wiatrowski, H.A., P.M. Ward, and T. Barkay. 2006. Novel reduction of mercury(II) by mercury-sensitive dissimilatory metal reducing bacteria. *Env. Sci. Technol.* **40**:6690-6696

Golding, G.R., C. A. Kelly, R. Sparling, P. C. Loewen, and T. Barkay. 2007. Evaluation of mercury toxicity as a predictor of mercury bioavailability. *Env. Sci. & Technol.* **41**:5685-5692

Wiatrowski, H.A. S. Das, R. Kukkadapu, E. Ilton, T. Barkay, and N. Yee. 2009. Reduction of Hg(II) to Hg(0) by Magnetite. *Env. Sci & Technol.* **42**:5307-5313

II. Conference presentations

Barkay T, Schaefer, J, Poulain, A. and, Amyot M. Microbial transformations in the mercury geochemical cycle. 15th Goldschmidt Conference. Moscow, Idaho, May 20–25, 2005. Invited talk

Wiatrowski, H. A., and T. Barkay. A novel mechanism for reduction of mercury (II) by *Shewanella oneidensis* MR-1. 105th Annu. Meet. Am. Soc. Microbiol. Atlanta, June 5–9, 2005.

Schaefer, J., and T. Barkay. Diversity of mercuric reductase (MerA) genes and transcripts in mercury contaminated waters. 105th Annu. Meet. Am. Soc. Microbiol. Atlanta, June 5–9, 2005.

Wiatrowski, H., and T. Barkay. Reduction of Hg(II) to Hg(0) by dissimilatory metal reducing bacteria. 8th International Conference on Mercury as a Global Pollutant. Madison, WI, Aug. 11–Aug 16, 2006.

Øregaard, G., J.R. de Lipthay, T. Barkay, and S.J. Sørensen. High diversity of bacterial mercuric reductase genes from surface and sub-surface soil. 11th International Symposium on Microbial Ecology. Viena, Austria, Aug. 20–25, 2006

Wang, Y., H.A. Wiatrowski, R. John, P. Lu-Irving, L. Young, L. Kerkhof, and T. Barkay. Impact of mercury on denitrifying microbial communities in subsurface sediments. 109th Annu. Meet. Am. Soc. Microbiol. Philadelphia, May 17 – 21, 2009

Ziogaite, B., B. Smith, A. Chatziefthimiou, T. Barkay, and J. Coombs. Isolation and Microarray Analysis of Cryptic Plasmids from Metal Resistant Bacteria of the Deep Terrestrial Subsurface. 109th Annu. Meet. Am. Soc. Microbiol. Philadelphia, May 17 – 21, 2009

Chatziefthimiou, A.D. A.L. Isola, and T. Barkay. Mercury Contamination and its Effects on Phylogenetic and Functional Diversity of Soil Hg^R Bacteria. 109th Annu. Meet. Am. Soc. Microbiol. Philadelphia, May 17 – 21, 2009