# Reduction of mercury by dissimilatory metal reducing bacteria

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## ABSTRACT

*Shewanella oneidensis* MR-1 is a facultative anaerobe that is of great interest for bioremediation due to its ability to reduce toxic metals and radionuclides. We present evidence that MR-1 can reduce ionic mercury [Hg(II)] to elemental mercury [Hg(0)] by a novel pathway that is not related to the well-characterized *mer* system. Additionally, MR-1 does not display Hg(II) resistance typical of an organism with a *mer* operon. In the presence of 150 nM Hg(II), MR-1 was able to reduce 64.8±9.13% of the Hg(II) in aerobic conditions, and 67.7±3.7% of the Hg(II) in fumarate reducing conditions. 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 ($p > 0.01$). This activity was constitutive , 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 Hg(II)/min/ mg protein respectively. Hg(II) reduction by MR-1 was enhanced five fold in iron reducing conditions relative to fumarate reducing conditions. This activity is not restricted to MR-1, as two other dissimilatory metal reducing bacteria (DMRB) which lack a *mer* system, *Geobacter sulfurreducens* PCA and *Geobacter metallireducens* GS-15, also are able to reduce Hg(II). Live and autoclaved cells of strain PCA had specific activities of 2.8±1.3 nmol Hg(II)/min/mg protein 0.34± 0.5 nmol Hg(II)/min/mg protein, respectively. For strain GS-15, live and autoclaved cells had specific activities of 6.05±1.4 nmol Hg(II)/min/mg protein and 1.65 ± 0.9 nmol Hg(II)/min/mg protein, 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. The discovery of constitutive reduction of Hg(II) at low concentrations by DMRB may have future applications in the remediation of anoxic soils and sediments.

## MATERIALS AND METHODS

- Mercury was analyzed using Cold Vapor Atomic Absorbance Spectroscopy or by Liquid Scintillation Counting using $^{203}Hg$ as a tracer.
- *S. oneidensis* was grown in M1 medium using lactate (10mM) as an electron donor and fumarate (10mM), ferric citrate (10mM) or ferric oxyhydroxide (10mM) as electron acceptors.
- *Geobacter metallireducens* GS-15 was grown in ATCC medium 1768 and *Geobacter sulfurreducens* PCA was grown in ATTC medium 1957 with ferric citrate (20mM) as an electron acceptor.
- In all experiments for which electron donating or electron accepting conditions varied from the culture conditions, cells were washed two times in media containing no electron donor and/or electron acceptor. Care was taken to ensure that anoxic conditions were maintained throughout the washes.
- Unless otherwise noted, Hg(II) was added as $HgCl_2$ at a concentration of 300nM.
- Cell concentrations were normalized to extractable protein, and a typical inoculum was ~ 0.4 μg protein/ml, which corresponds to approximately $10^5$ cells/ml
- Data represents means of three replicates, and all errors are standard deviations. Means that are not significantly ($p > .05$) are indicated with the same letter or number. Means that are highly significantly different ($p < .01$) are indicated with an asterisk.

## ACKNOWLEDGEMENTS

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## RESULTS

### Reduction of Hg(II) by MR-1

**Fig 1: MR-1 causes loss of Hg(II) from culture media**

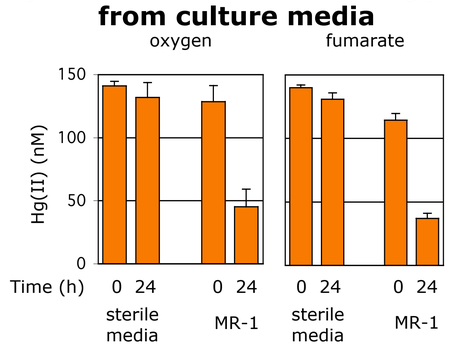
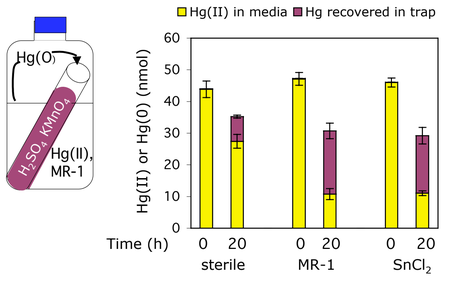



**Fig 2: Mercury is lost as elemental mercury**




[Hg(II)] = 650nM, [$SnCl_2$] = 15mM

### Comparing Hg(II) reduction by MR-1 and the *mer* system

**To facilitate comparison, a *mer* system was introduced to MR-1**

- A spontaneous rifampicin resistant mutant was selected for. This strain was called MR-1H
- This strain was mated to *Psuedomonas aeruginosa* PAO-1 containing a *mer* system on the plasmid R388::*Tn501,* generating a transconjugate called MR-1H/R388::*Tn501*

**MR-1 is not resistant to mercury**

- MR-1 is inhibited by 0.5 μM Hg(II)
- MR-1H/R388::*Tn501* can grow in the presence of 25 μM Hg(II)

***mer*-independent reduction is not an inducible process.**

- Cells pregrown in 300nM Hg(II) and unexposed cells had specific activities of 3.14±0.25 and 3.07± 0.35 nmol Hg(II)/min/mg protein respectively.

**Table 1: The *mer* system reduces Hg(II) at high concentrations but is inefficient at low concentrations, while *mer* independent reduction occurs at low concentrations**

| | 25 μM $HgCl_2$ | | 300 nM $HgCl_2$ | |
|---|---|---|---|---|
| Strain | percent Hg lost at 25h | rate of reduction (nmol/min/ mg protein) | percent Hg lost at 24h | initial rate of reduction (nmol/min/mg protein$^{-1}$) |
| MR-1H R388::*Tn501* | 48.0 ± 8.4 A* | 13.5 ± 2.6 A* | 69.8 ± 3.5 A* | 1.06 ± 0.32 A* |
| MR-1H | 6.0 ± 1.3 B | 1.2 ± 0.7 B | 44.3 ± 2.4 B* | 0.44 ± 0.08 B |
| MR-1 | 5.9 ± 1.7 B | 2.0 ± 0.6 B | ND | 2.56 ± 0.17 C* |
| MR-1H, autoclaved | 4.2 ± 2.3 B | 0.7 ± 0.4 B | 20.4 ± 3.4 C | 0.28 ± 0.04 B |
| uninoculated media | 3.4 ± 3.1 B | 0.8 ± 1.0 B | 15.2 ± 6.0 C | 0.20 ± 0.08 B |

### Cell density dependence of Hg(II) reduction by MR-1

**Figure 3: As cell density increases, rate and specific activity of Hg(II) reduction decreases**

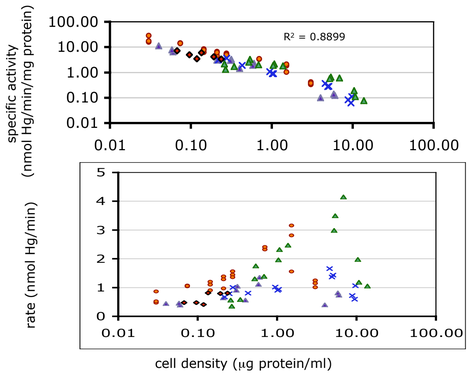
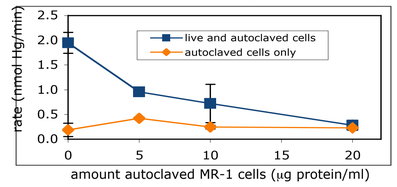



**Figure 4: Cellular material inhibits reduction rates by sequestering Hg(II)**




### Interactions between Hg(II) reduction and growth conditions

**Figure 5: Electron donors and electron acceptors are required for Hg(II) reduction by MR-1**

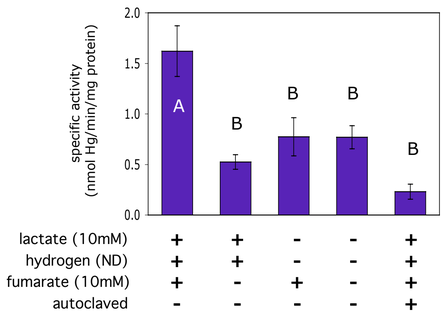
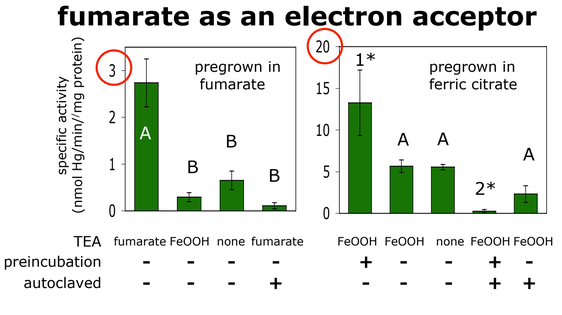


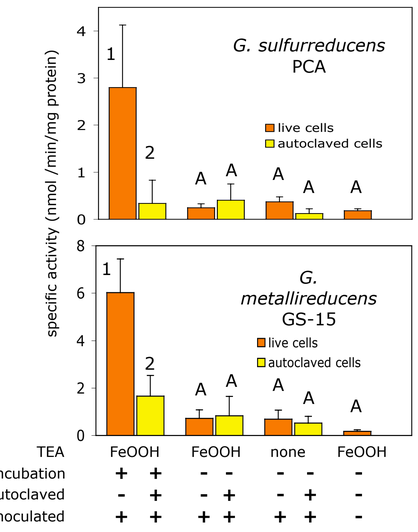

**Figure 6: A higher reduction rate of Hg(II) occurs with iron as compared to fumarate as an electron acceptor**




- MR-1 reduces Hg(II) at ~5 X higher rate in iron versus fumarate reducing conditions
- A 24h preincubation period in FeOOH was required for Hg(II) reduction activity. No growth was observed during this time.

### Hg(II) reduction by other DMRB

**Figure 7: *Geobacter spp.* reduce Hg(II)**




Cells were pregrown with ferric citrate as a terminal electron acceptor. Both species require an overnight incubation in FeOOH to reduce Hg(II).

No reduction of Hg(II) was observed by *Anaeromyxobacter dehalogenans* 2CP-C after 1 week, despite apparent growth.

## CONCLUSIONS

- *S. oneidensis* MR-1, *G. sulfurreducens* PCA, and *G. metallireducens* GS-15 are able to reduce Hg(II). (Figs. 1, 2, 7)
- This reduction is not as effective as the *mer* system (Table 1) and does not provide resistance to Hg(II), but it is most effective at low cell densities (Figs. 3, 4) and at low Hg(II) concentrations (Table 1) *mer*-independent reduction, unlike *mer,* is not an inducible process
- *mer*-independent reduction of Hg(II) requires presence of an electron donor and acceptor (Fig.5), occurs in aerobic conditions (Fig 1), fumarate reducing conditions (Figs. 1, 2, 5, 6) and iron reducing conditions, and is enhanced in iron reducing conditions (Fig. 6)
- We hypothesize that MR-1 reduces Hg(II) through its electron transport chain.

## ENVIRONMENTAL RELEVANCE

- Many DOE sites are contaminated with low levels of Hg(II), possibly too low to induce the *mer* operon. Because reduction by MR-1 is constitutive and functions at low Hg(II) concentrations, it may be more applicable to these environments than *mer*-mediated reduction.
- Reduction of Hg(II) to Hg(0) increases its mobility, because Hg(II) sorbs to sediments and Hg(0) is a volatile gas that is poorly soluble in water.
- Reduction of Hg(II) to Hg(0) may make it less available for methylation by sulfate reducing bacteria when biostimulation is used to immobilize radionuclides.