Microbial Pathways for the Reduction of Mercury in Saturated Subsurface Sediments

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ABSTRACT

Mercury in contaminated subsurface soils may be leached to the saturated zone where its reduction to the elemental form, Hg(0), may enhance its environmental mobilization. Therefore, microbial transformations that reduce the mercuric ion, Hg(II), to Hg(0) are of key importance to the remediation of mercury in contaminated subsurface sediments. Microbes may reduce Hg(II) by the activity of the inducible mercuric reductase (MR), the gene product of *merA*, and this process is well understood in a broad range of aerobic bacteria and environments. Little is known about Hg(II) reduction in anoxic environments. We therefore have initiated a study on the presence of *merA* in microbial communities of anoxic environments and the effect of anaerobic respiratory pathways on MR expression and activities. PCR primers were designed to span the known phylogenetic range of *merA* genes of Gram-negative bacteria. In control experiments, these primers successfully amplified a 288 bp region at the 3' end of previously characterized *merA* genes from *Shewanella putrefaciens* pMERPH, *Acidithiobacillus ferrooxidans*, *Pseudomonas stutzeri* ppB, Tn*5041*, *Pseudomonas* sp. K-62, and *Serratia marcescens* pDU1358. The abundance and diversity of *merA* were assessed in anaerobic enrichments from Berry's Creek, a highly industrially contaminated site in the Meadowlands, NJ, by sequencing a *merA* clone library obtained by PCR from sediment DNA extracts. Anaerobic sediment slurries were supplemented with two additions of 10 µg Hg(II)/g and incubated for 3 weeks at which time the DNA was isolated and PCR amplified. The amplicons were TOPO cloned into pCR2.1 (Invitrogen) and sequenced. A total of 174 clones were sequenced of which 88 represented unique amplicons. The sequences were aligned and a phylogenetic tree was built. While many sequences aligned with previously described *merA* genes from both Gram positive and Gram negative isolates, four novel lineages (II through V), were identified. These results reveal a previously unrecognized diversity of *merA* and suggest that bacterial activities may play a role in mercury reduction in anaerobic environments.

Anaerobic enrichments of Meadowlands sediments were set up for the purpose of isolating pure cultures carrying novel *merA* genes. Five oligoprobes specific for each of the novel *merA* cluster were designed, tested, and hybridized with genomic DNA of Hg(II) resistant isolates from the enrichments. Three strains, a *Bacillus* sp. and a *Streptomyces* sp. with *merA* of cluster V, and a *Pseudomonas* sp. with *merA* of cluster II, were isolated from the fermentative enrichment. One denitrifying isolate, a *Paenibacillus* sp. carried a cluster III *merA*. The entire mercury resistance systems in these strains are currently being examined genetically and biochemically to fully characterize the mechanisms by which anaerobic bacteria interact with mercury. Similar studies are being set up with subsurface sediments from mercury contaminated and control aquifers.

A PCR approach to encompass the known diversity of MerA in gram negative bacteria

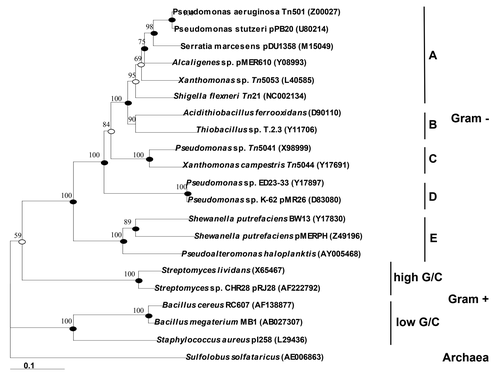


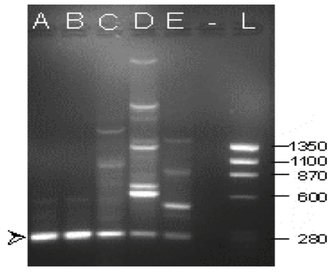
Figure 1: Neighbor-joining (NJ) tree showing the phylogenetic relationship of complete MerA sequences from representative Hg resistant microorganisms. Clades A–E within the Gram negative bacteria are indicated on the tree. Bootstrap values for the NJ tree are shown at each branch point. Nodes also supported by parsimony analysis (heuristic search) with bootstrap values >74 are shown as closed circles. Branch points supported by NJ but not parsimony analysis are shown as open circles. The bar represents 0.1 nucleotide substitution per site. NCBI accession numbers are indicated in parentheses.

merA degenerate primers:

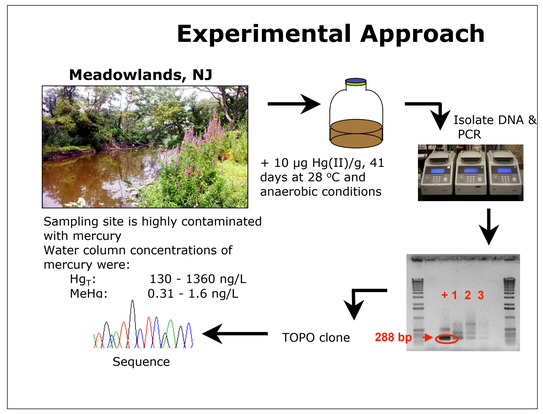
Forward primer A1s-n.F: 5'-TCC GCA AGT NGC VAC BGT NGG-3'
Reverse primer A5-n.R: 5'-ACC ATC GTC AGR TAR GGR AAV A-3'

Figure 2: Gel electrophoresis showing *merA* amplification products from reference strains.

Lane designations correspond to clades in Figure 1. A, pDU1358; B, *Acidithiobacillus ferrooxidans*; C, *Tn*5041; D, *Pseudomonas* sp. K-62; E, pMERPH; –, no DNA control; and L, fX174 *Hae*III (Fisher). Arrow shows the expected ~288 bp product.



High diversity and novel clusters in a *merA* clone library from mercury contaminated sediments



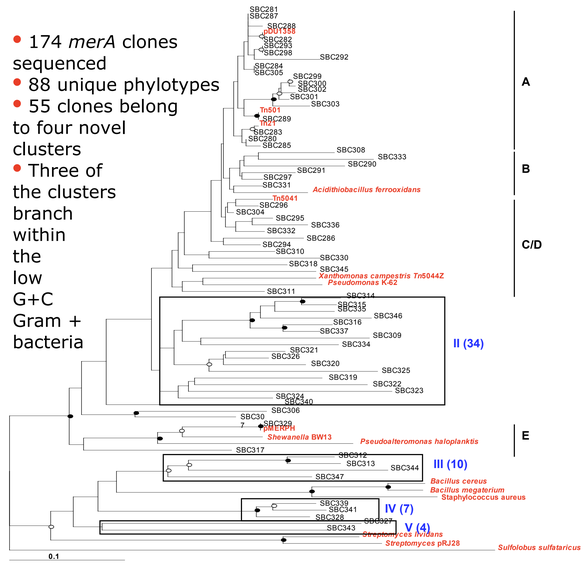


Figure 3: NJ tree of derived amino acid sequences from *merA* PCR products (~244 bp) obtained from Berry's Creek sediment. Numbers in parentheses following clade designations indicate the number of clones within that clade. Unique clades (II – V) detected in this library are outlined in boxes.

Enrichment cultures belonging to novel *merA* clades were isolated

- **Pure cultures of Hg(II) resistant bacteria were isolated from Meadowlands sediment enrichments under fermentative or denitrfication conditions**
- **Genomic DNA was hybridized to oligoprobes designed for unique clades II - V**
- ***mer* operons from strains belonging to *merA* clades II, III, and V are currently analyzed**

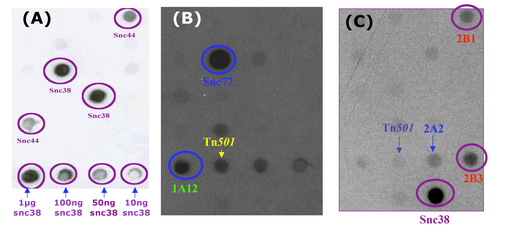


Figure 4: DNA hybridization signals with biotin-labeled probes for unique *merA* clades **(A)** Specificity of the probe to clade V. **(B)** Identification of enrichment culture 1A12 as belonging to *merA* clade II. **(C)** Identification of enrichment cultures 2B1 and 2B3 as belonging to *merA* clade V.

Cultures currently under study are:

Taxon of closest relative	Clade	16S rRNA similarity
Pseudomonas sp. 1A12	II	99%
Bacillus sp 2B1	V	97%
Streptomyces sp. 2B3	V	98%
Paenibacillus sp. N10	III	97%

CONCLUSIONS

- **An experimental approach and methods for the study of the diversity of genes encoding for mercuric reductase enzymes in environmental microbial biomaass are available**
- **A high, and hitherto unrecognized, diversity of merA was found in the microbial community of a contaminated anoxic sediment**
- **This study will be expanded to the microbial biomass of subsurface sediments**