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Analysis of mercuric reductase (*merA*) gene diversity in an anaerobic mercury-contaminated sediment enrichment

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Summary

The reduction of ionic mercury to elemental mercury by the mercuric reductase (MerA) enzyme plays an important role in the biogeochemical cycling of mercury in contaminated environments by partitioning mercury to the atmosphere. This activity, common in aerobic environments, has rarely been examined in anoxic sediments where production of highly toxic methylmercury occurs. Novel degenerate PCR primers were developed which span the known diversity of *merA* genes in Gram-negative bacteria and amplify a 285 bp fragment at the 3′ end of *merA*. These primers were used to create a clone library and to analyse *merA* diversity in an anaerobic sediment enrichment collected from a mercury-contaminated site in the Meadowlands, New Jersey. A total of 174 sequences were analysed, representing 71 *merA* phylotypes and four novel MerA clades. This first examination of *merA* diversity in anoxic environments suggests an untapped resource for novel *merA* sequences.

Introduction

Mercury contamination is a widespread environmental problem due to atmospheric transport and deposition and to point sources of pollution. Biogeochemical cycling plays a critical role in determining mercury toxicity because mercury is primarily deposited in the environment as ionic mercury, Hg(II), where it may be methylated to the neurotoxic substance methylmercury (MeHg). Methylmercury, following its bioaccumulation and biomagnification in food chains, poses a risk to consumers at the upper trophic levels. Thus, microbial transformations that directly or indirectly affect the rates of Hg(II) methylation or MeHg degradation play a critical role in modulating mercury toxicity. The prokaryotic mercury resistance mechanism plays a unique role in this paradigm by degrading MeHg and reducing Hg(II) to volatile elemental mercury, Hg(0) (Barkay *et al*., 2003; Barkay and Wagner-Döbler, 2005).

In bacteria and possibly archaea (Schelert *et al*., 2004), mercury resistance is specified by mercury resistance (*mer*) operon functions that transport Hg(II) and organomercury compounds to the cytosol for degradation and reduction to Hg(0). The latter is catalysed by the mercuric reductase enzyme (MerA), a flavin oxidoreductase which reduces Hg(II) in an NAD(P)H-dependent reaction. Microbial communities acclimate to life in the presence of mercury through: (i) enrichment of resistant organisms (Barkay, 1987; Rasmussen and Sørensen, 1998; 2001; Müller *et al*., 2001); (ii) induction and synthesis of *mer* gene products (Nazaret *et al*., 1994; Schaefer *et al*., 2004); and possibly (iii) horizontal gene transfer of *mer* determinants (Dronen *et al*., 1998; Rasmussen and Sorensen, 1998; Smit *et al*., 1998). In environments inhabited by acclimated communities, the enrichment and activities of resistant microbes may play a significant role in mercury biogeochemistry by degrading MeHg and reducing Hg(II). This is supported by correlations between MerA activity (Siciliano *et al*., 2002) and transcript abundance (Schaefer *et al*., 2004) and the flux of Hg(0) to the atmosphere from natural waters.

Little is at present known about Hg(II)-resistant microbes and MerA activities in anaerobic environments. Yet, it is there that MerA activities can most significantly affect MeHg production by competing for Hg(II) with methylating microbes, mostly sulfate-reducing bacteria (Benoit *et al*., 2003). MerA and its activities were documented among strict anaerobes (Rudrick *et al*., 1985) and the formation of Hg(0) in anoxic sediment incubations has been demonstrated (Weber *et al*., 1998). Schaefer and colleagues (2002) compared MerA induction and activities

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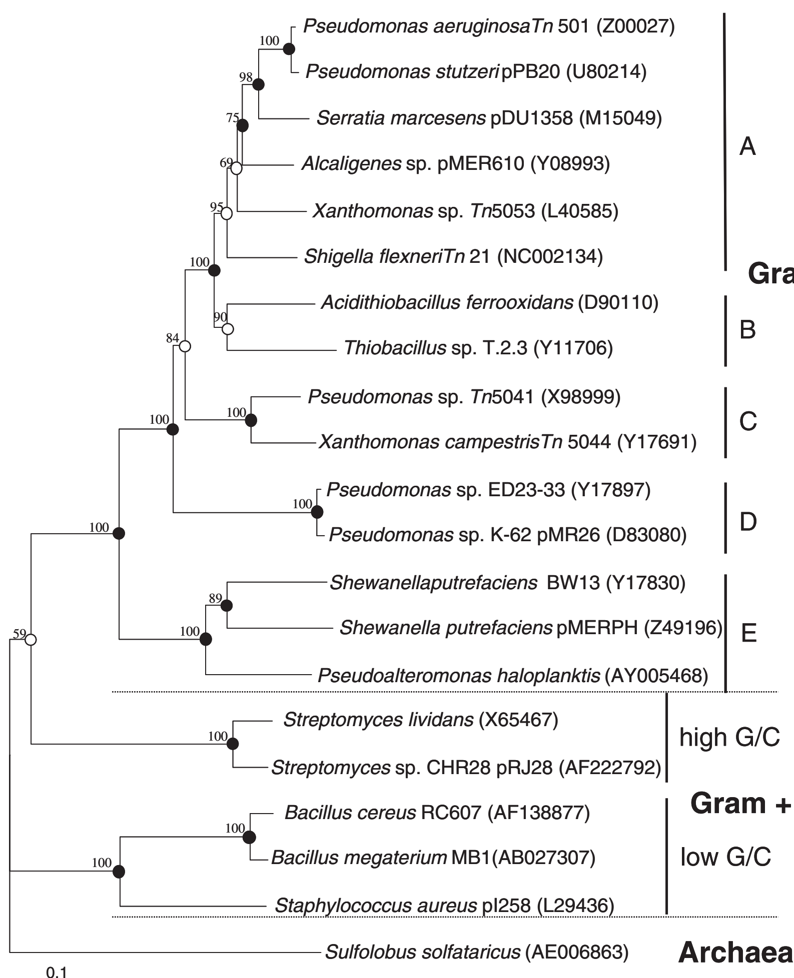

Fig. 1. Neighbour-joining (NJ) tree (CLUSTALX [v. 1.8.1]; Thompson *et al.*, 1997) showing the phylogenetic relationship of complete MerA sequences among mercury-resistant prokaryotes that were obtained from GenBank. Clades A–E within the Gram-negative bacteria are marked. Bootstrap values (PAUP v. 4.0b10; Swofford, 1998) 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 amino acid substitutions per site. NCBI accession numbers are indicated in parentheses. The archaeal MerA of *Sulfolobus sulfataricus* served as an out group and the tree was drawn in TreeView (v. 1.6.6; Page, 1996).

under aerobic and anaerobic conditions using a denitrifying soil bacterium. Results showed that *mer* operon functions were induced at higher concentrations of Hg(II) anaerobically than aerobically, and induction patterns of a *mer-lacZ* gene fusion suggested that low redox resulted in reduced transport of Hg(II) into the cytosol (Schaefer *et al.*, 2002). Nevertheless, in highly contaminated sediments where resistant microbes are enriched, MerA-dependent reduction of Hg(II) may control MeHg production by substrate competition. If so, the diversity of MerA determinants in microbes that inhabit anoxic sediments is critical for sustained *mer* operon functions under varied environmental conditions.

Here we employed new degenerate *merA* PCR primers, designed to target the known diversity of *merA* sequences among Gram-negative bacteria, to describe the diversity of MerA in the microbial community of an anaerobic enrichment that originated in a sediment sample from a mercury-contaminated stream. Our results reveal a hitherto unappreciated diversity of *merA* in anoxic environments.

Results and discussion

MerA diversity and design of PCR primers

Phylogenetic analysis of MerA sequences from representative strains showed distinct separation among Archaea, low G/C and high G/C Gram-positive bacteria, and Gram-negative bacteria. Within the Gram-negative bacteria, *merA* sequences formed five major clades designated A–E (Fig. 1). Because previously developed primers (Liebert *et al.*, 1997; Felske *et al.*, 2003) had only targeted Gram-negative *merA*s from Clades A and B (Fig. 1) the design and testing of new PCR primers that encompassed a broader diversity of MerA was undertaken. Alignment of representative MerAs from Gram-negative bacteria revealed highly conserved regions at the C-terminal of the protein suitable for the design of degenerate PCR primers. Primers were designed to this conserved region (amino acids 444–451 and 532–539), in the pyridine nucleotide disulfide oxidoreductase dimerization domain of Tn*501* MerA (Venkatesan *et al.*, 2001). These primers, A1s-n.F and A5-n.R (see legend to Fig. 2 for

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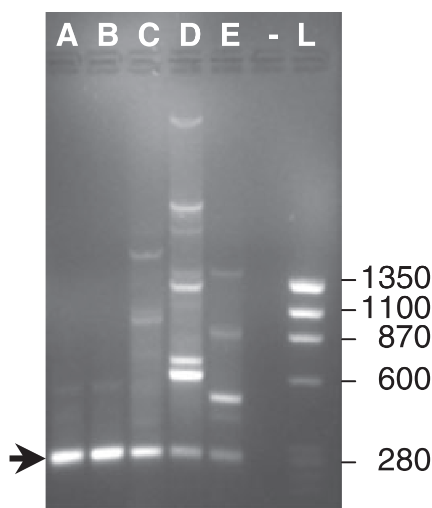

Fig. 2. Gel electrophoresis showing the specificity of a newly designed PCR primer set for *merA* in Gram-negative bacteria. PCR reactions were prepared in either 25 or 50 μl volumes containing 1× PCR buffer, 0.8 μM each forward (A1s-n.F: TCCGCAAGTNGCVACBGTNGG) and reverse (A5-n.R: ACCATCGTCAGRTARGGRAAVA) primers, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.025 U μl^{-1} Taq Polymerase (USB, Cleveland, OH) and 50–100 ng DNA template. Amplifications were as follows: an initial denaturation step of 94°C for 5 min, 45 cycles of 94°C for 10 s, 54°C for 60 s, and 72°C for 60 s, followed by a final extension of 72°C for 7 min. Target DNA carrying reference *merA*s included loci originated in (lane designations correspond to clades as depicted in Fig. 1): *Serratia marcescens* pDU1358 (Griffin *et al*., 1987), *Acidithiobacillus ferrooxidans* (Inoue *et al*., 1989), *Pseudomonas* sp. *Tn*5041 (Kholodii *et al*., 1997), *Pseudomonas* sp. K-62 pMR26 (Kiyono *et al*., 1997), and *Shewanella putrefaciens* pMERPH (Osborn *et al*., 1996); –, no DNA control; L, size marker – ϕX174 HaeIII (Fisher Scientific). The sizes of marker bands are shown on the right. Arrow points to the expected 285 bp product.

details), amplified the expected 285 bp gene fragment from *merA* representing Clades A–E (Fig. 2). The specificity of the PCR reactions varied with numerous additional PCR products observed with templates from clades C–E suggesting that the primers annealed more specifically to *merA* in clades A and B. Numerous attempts at optimization of PCR conditions by varying annealing temperatures and $MgCl_2$ concentrations did not result in the elimination of these extra bands. However, when A1s-n.F and A5-n.R were used with genomic DNA of pure cultures (Vetriani *et al*., 2005) or environmental DNA extracts (Schaefer *et al*., 2004) as templates the 285 bp was usually the sole PCR product obtained.

MerA clone library from a sediment enrichment

The new *merA* PCR primers were used to examine the diversity of *merA* in an anaerobic sediment enrichment. A single bulk sample was collected manually from Berry's Creek, a tributary of the Hackensack River in the Meadowlands, NJ, USA, in April, 2003. Total mercury concentrations in the vicinity of the sampled site ranged from 13 to 917 $\mu g\ g^{-1}$ wet weight sediment (T. Cardona-Marek *et al*., in preparation) indicating a high degree of contamination considering that the average earth crust concentration of mercury is 0.5 $\mu g\ g^{-1}$ (Weast, 1973). The sample was stored under anaerobic conditions at 4°C, following sampling. An anaerobic sediment enrichment was set up within days of sampling. A sediment slurry was prepared in a 125 ml serum bottle by mixing 50 g sediment and 50 ml N_2-flushed and deionized water and sealed with a Teflon stopper under O_2-free nitrogen gas. The enrichment was supplemented with 10 μg Hg(II) per ml slurry (as $HgCl_2$) on days 0 and 5 and incubated at 28°C. After 41 days, total genomic DNA was isolated from 10 replicates of 5 ml slurries using the UltraClean Soil DNA Isolation Kit (MoBio, Solana Beach, CA) and the resulting DNA extracts were combined and used as template for *merA* PCR amplification and clone library construction. Attempts to amplify *merA* from DNA extracts of the same sediment prior to enrichment were unsuccessful. Gel purified (QIAquick gel extraction kit, Qiagen, Valencia, CA) 285 bp *merA* amplification products were cloned (TOPO-TA cloning kit, Invitrogen, Carlsbad, CA) and inserts of the expected size were sequenced on an ABI 3100 Genetic Analyzer using either the M13f or M13r primers and ABI dye terminator chemistry (BigDye v. 3.1, Applied Biosystems, Foster City, CA) on an ABI 3100 Genetic Analyzer. The 174 *merA* clones that were sequenced yielded 71 *merA* phylotypes using a phylotype definition of 98% sequence identity. Sequences related to all five Gram-negative clades were found but those that were grouped with Clade A dominated, comprising 50% of all clones sequenced (Fig. 3). Four unique phylotype groups (representing 30% of the clones sequenced) were not related to any known reference sequence. Most interestingly, three of these novel groups (I–III) clustered with MerA sequences from the low G + C Gram-positive bacteria. Clones from these groups accounted for 12% of the entire library and were most closely related to MerA's of *Bacillus licheniformis*, *Geobacillus kaustophilus, Bacillus macroides* and *Bacillus sphaericus* with identities ranging from 46% to 61%. The fourth novel group (IV) contained 12 phylotypes and accounted for 15% of the library and was most closely related to MerA of *Tn*5041 (80% identity) a member of Clade C (Fig. 1).

This first description of *merA* in microbial biomass from contaminated sediment raises the question of whether or not MerA is expressed in anoxic environments. Observations implicating an oxidative pathway, rather than a reductive, *mer* specified pathway, in the degradation of MeHg in Meadowlands sediments (T. Cardona-Marek *et al*., in preparation) suggest that if MerA is expressed its role in mercury cycling in these sediments may be limited.

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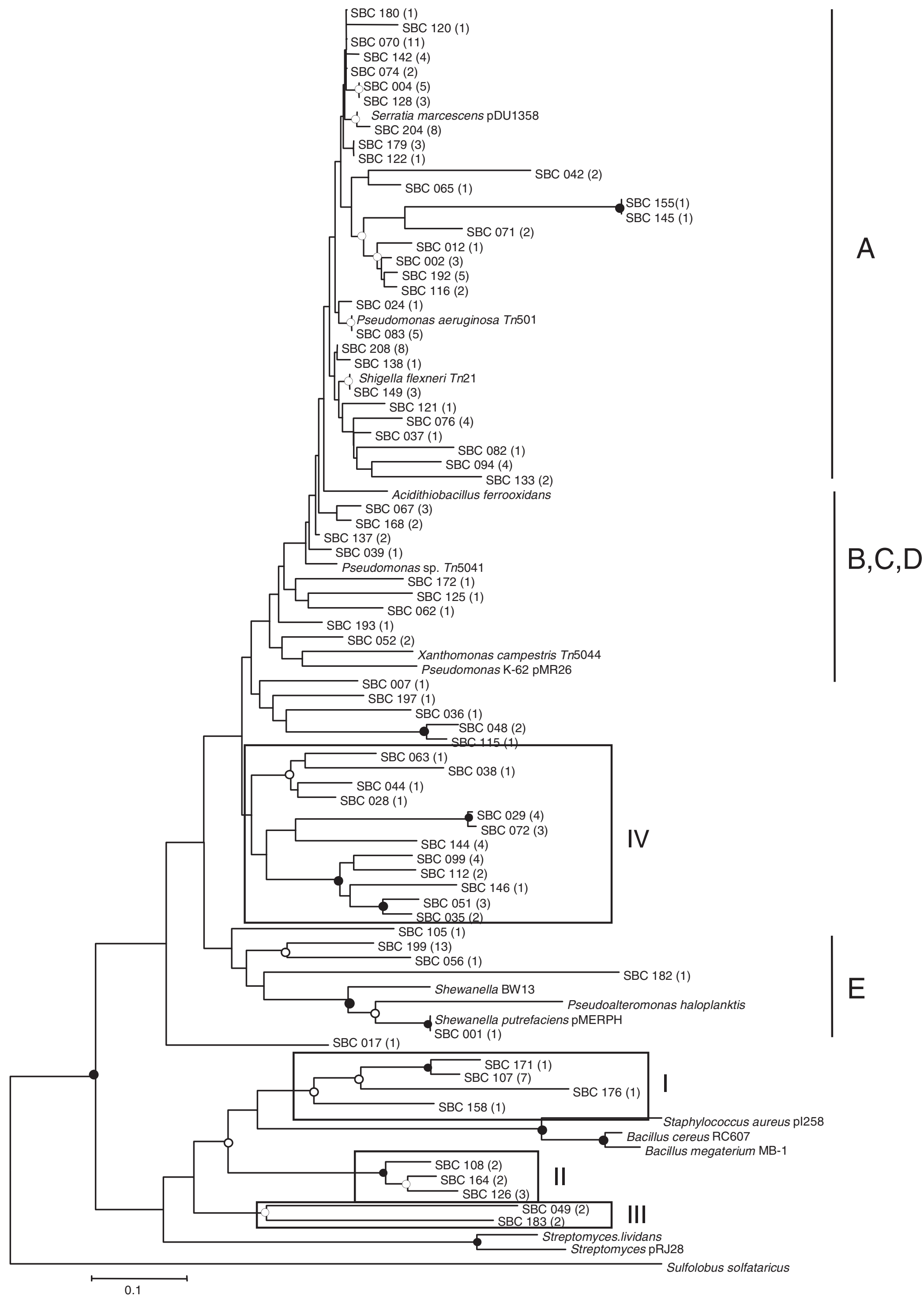

Fig. 3. Neighbour-joining (NJ) tree of derived amino acid sequences from trimmed *merA* PCR products (239–254 bp) obtained from Berry's Creek sediment. Sequence homology was verified using BLASTX (Altschul *et al.*, 1990) and alignments and tree building were performed as described in the legend to Fig. 1. Numbers in parentheses after the sequence designation indicate the number of clones within that phylotype. Bootstrap values > 50 and > 75 are indicated at each branch node as open and closed circles respectively. Unique clades (I–IV) detected in this library are outlined in boxes. The bar represents 0.1 amino acid substitutions per site. The nucleotide sequences described in this report have been deposited in GenBank under accession numbers DQ132515–DQ132604.

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Nevertheless, the discovery of diverse *merA* loci in the microbial biomass of a contaminated sediment identifies a need for further examination of the role played by MerA in mercury cycling in the environment where bacterial MeHg production occurs (Compeau and Bartha, 1985; Gilmour *et al.*, 1992).

Diversity analysis of merA *gene sequences*

Several tests were applied to the sediment *merA* clone library to estimate sampling efficiency by rarefaction analysis (Hughes *et al.*, 2001) and to calculate non-parametric richness estimates ACE (Chao and Lee, 1992) and Chao1 (Chao, 1984) as a function of sampling effort from rarefaction curves following 1000 randomizations. A distance matrix of aligned *merA* sequences was generated using the F84 model for nucleotide substitution using the DNADIST program in PHYLIP (Felsenstein, 2004). This distance matrix was then used to calculate sample coverage and richness estimates in DOTUR (Schloss and Handelsman, 2005). Phylotypes were assigned using the furthest neighbour clustering algorithms.

A rarefaction curve for the sediment library did not reach a plateau (data not shown), suggesting that further sampling was needed to adequately describe the diversity of *merA* in the sediment community. This conclusion was supported by the Chao1 and ACE richness estimates that predicted 106 and 111 *merA* phylotypes respectively, while we observed 71 phylotypes in the sediment library indicating that sequenced clones captured approximately 75% of the diversity in the library.

Although the diversity analysis was conducted using a phylotype definition of 98% identity, a plot of Chao1 richness estimates versus per cent identity clearly illustrates that reducing the criterion used to define a phylotype for functional genes to a more conservative 94% identity, as has been reported previously (Venter *et al.*, 2004), does not significantly affect the richness estimate (Fig. 4). Lowering the phylotype definition to 76% identity reduced the richness estimate for the sediment community to 40. In contrast, when the 76% phylotype definition was applied to the reference sequences used to establish Fig. 1, sequences from Clades A, B and C (with the exception of the *Thiobacillus* sp. T.2.3 sequence) clustered into a single phylotype (data not shown). The sensitivity of clustering patterns to phylotype definition of *merA* of environmental clones relative to that of reference sequences further highlights the high divergence of the environmental *merA* clone library.

The high diversity detected among *merA* clones was unexpected as the sediment enrichment was established with sediment from a contaminated site where one would expect strong selection for specific loci. Because Berry's Creek sediment has been exposed to mercury for close to

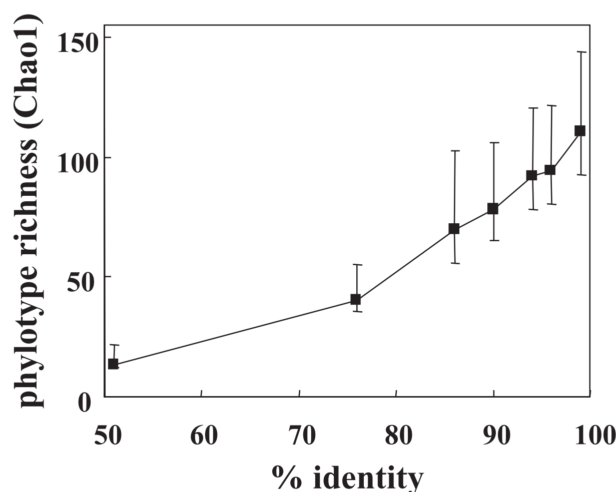

Fig. 4. Chao1 estimates of *merA* phylotype richness as a function of the percentage identity for the *merA* library [calculated using DOTUR (Schloss and Handelsman, 2005)]. Error bars represent lower and upper 95% confidence intervals.

70 years (Lipsky and Koepp, 1986) it is possible that the high *merA* sequence diversity observed reflects diversification of resistant microorganisms during prolonged evolution in a contaminated and heterogeneous environment. This observation highlights the need for further study of how the diversity of a locus such as *mer* is modulated in complex environments under selective pressure.

In summary, this study employed improved degenerate PCR primers to show a high diversity of *merA* genes in an environmental clone library that was constructed from a mercury-enriched sediment sample that originated in a mercury-contaminated stream. This diversity exceeded the diversity that is presently defined by MerA in cultured microbes or that could be defined using previously existing *merA* PCR primers (Liebert *et al.*, 1997; Felske *et al.*, 2003). We estimate that the improved primers enabled detection of an additional 37 phylotypes in the anaerobic sediment library that would have not been detected using previously published primers. Thus, these primers allow a significant improvement in assessing *merA* diversity in uncultured environmental samples. Gene sequences obtained with these primers will serve as a tool to monitor the potential for mercury reduction in the environment and will facilitate the isolation of microorganisms containing novel *merA* genes to allow an assessment of their activities in the laboratory and the environment.

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References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. *J Mol Biol* **215:** 403–410.

Barkay, T. (1987) Adaptation of aquatic microbial communities to Hg^{+2} stress. *Appl Environ Microbiol* **53:** 2725–2732.

Barkay, T., and Wagner-Döbler, I. (2005) Microbial transformations of mercury: potentials, challenges, and achievements in controlling mercury toxicity in the environment. *Adv Appl Microbiol* **57:** 1–52.

Barkay, T., Miller, S.M., and Summers, A.O. (2003) Bacterial mercury resistance from atoms to ecosystems. *FEMS Microbiol Rev* **27:** 355–384.

Benoit, J.M., Gilmour, C.C., Heyes, A., Mason, R.P., and Miller, C.L. (2003) Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. In *Biogeochemistry of Environmentally Important Trace Elements.* Chai, Y. and Braids, D.C. (eds). Washington, DC: American Chemical Society, pp. 262–297.

Chao, A. (1984) Non-parametric estimation of the number of classes in a population. *Scand J Stat* **11:** 265–270.

Chao, A., and Lee, S.M. (1992) Estimating the number of classes via sample coverage. *J Am Stat Assoc* **87:** 210–217.

Compeau, G.C., and Bartha, R. (1985) Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. *Appl Environ Microbiol* **50:** 498–502.

Dronen, A.K., Torsvik, V., Goksoyr, J., and Top, E.M. (1998) Effect of mercury addition on plasmid incidence and gene mobilizing capacity in bulk soil. *FEMS Microbiol Ecol* **27:** 381–394.

Felsenstein, J. (2004) PHYLIP (Phylogeny Inference Package), version 3.6.: Department of Genome Sciences, University of Washington, Seattle, Distributed by the author.

Felske, A.D.M., Fehr, W., Pauling, B.V., von Canstein, H., and Wagner-Döbler, I. (2003) Functional profiling of mercuric reductase (*mer A*) genes in biofilm communities of a technical scale biocatalyzer. *BMC Microbiol* **3:** 22.

Gilmour, C.C., Henry, E.A., and Mitchell, R. (1992) Sulfate stimulation of mercury methylation in freshwater sediments. *Environ Sci Technol* **26:** 2281–2287.

Griffin, H.G., Foster, T.J., Silver, S., and Misra, T.K. (1987) Cloning and DNA sequence of the mercuric- and organomercurial- resistance determinants of plasmid pDU1358. *Proc Natl Acad Sci USA* **84:** 3112–3116.

Hughes, J.B., Hellmann, J.J., Ricketts, T.H., and Bohannan, B.J.M. (2001) Counting the uncountable: statistical approaches to estimating microbial diversity. *Appl Environ Microbiol* **67:** 4399–4406.

Inoue, C., Sugawara, K., Shiratori, T., Kusano, T., and Kitagawa, Y. (1989) Nucleotide sequence of the *Thiobacillus ferrooxidans* chromosomal gene encoding mercuric reductase. *Gene* **84:** 47–54.

Kholodii, G.Y., Yurieva, O.V., Gorlenko, Z.M., Mindlin, S.Z., Bass, I.A., Lomovskaya, O.L., *et al.* (1997) Tn*5041*: a chimeric mercury resistance transposon closely related to the toluene degradative transposon Tn*4651*. *Microbiology* **143:** 2549–2556.

Kiyono, M., Omura, T., Inuzuka, M., Fujimori, H., and Hidemitsu, P.H. (1997) Nucleotide sequence and expression of the organomercurial-resistance determinants from a *Pseudomonas* K-62 plasmid pMR26. *Gene* **189:** 151–157.

Liebert, C.A., Wireman, J., Smith, T., and Summers, A.O. (1997) Phylogeny of mercury resistance (*mer*) operons of gram-negative bacteria isolated from the fecal flora of primates. *Appl Environ Microbiol* **63:** 1066–1076.

Lipsky, D.S., and Koepp, S.J. (1986) Mercury levels in organisms in proximity to an old chemical site (Berry's Creek, Hackensack Meadowlands, New Jersey, USA). *Mar Pollut Bull* **17:** 219–224.

Müller, A.K., Westergaard, K., Christensen, S., and Sorensen, S.J. (2001) The effect of long-term mercury pollution on the soil microbial community. *FEMS Microbiol Ecol* **36:** 11–19.

Nazaret, S., Jeffrey, W.H., Saouter, E., Vonhaven, R., and Barkay, T. (1994) *merA* gene expression in aquatic environments measured by mRNA production and Hg(II) volatilization. *Appl Environ Microbiol* **60:** 4059–4065.

Osborn, A.M., Bruce, K.D., Ritchie, D.A., and Strike, P. (1996) The mercury resistance operon of the IncJ plasmid pMERPH exhibits structural and regulatory divergence from other Gram-negative mer operons. *Microbiology* **142:** 337–345.

Page, R.D.M. (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12:** 357–358.

Rasmussen, L.D., and Sørensen, S.J. (1998) The effect of longterm exposure to mercury on the bacterial community in marine sediment. *Curr Microbiol* **36:** 291–297.

Rasmussen, L.D., and Sørensen, S.J. (2001) Effects of mercury contamination on the culturable heterotrophic, functional and genetic diversity of the bacterial community in soil. *FEMS Microbiol Ecol* **36:** 1–9.

Rudrick, J.T., Bawdon, R.E., and Guss, S.P. (1985) Determination of mercury and organomercurial resistance in obligate anaerobic bacteria. *Can J Microbiol* **31:** 276–281.

Schaefer, J.K., Letowski, J., and Barkay, T. (2002) *mer*-mediated resistance and volatilization of Hg(II) under anaerobic conditions. *Geomicrobiol J* **19:** 87–102.

Schaefer, J.K., Yagi, J., Reinfelder, J.R., Cardona, T., Ellickson, K.M., Tel-Or, S., and Barkay, T. (2004) Role of the bacterial organomercury lyase (MerB) in controlling methylmercury accumulation in mercury-contaminated natural waters. *Environ Sci Technol* **38:** 4304–4311.

Schelert, J., Dixit, V., Hoang, V., Simbahan, J., Drozda, M., and Blum, P. (2004) Occurrence and characterization of mercury resistance in the hyperthermophilic archaeon *Sulfolobus solfataricus* by use of gene disruption. *J Bacteriol* **186:** 427–437.

Schloss, P.D., and Handelsman, J. (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microbiol* **71:** 1501–1506.

Siciliano, S.D., O'Driscoll, N.J., and Lean, D.R.S. (2002) Microbial reduction and oxidation of mercury in freshwater lakes. *Environ Sci Technol* **36:** 3064–3068.

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Smit, E., Wolters, A., and van Elsas, J.D. (1998) Self-transmissible mercury resistance plasmids with gene-mobilizing capacity in soil bacterial populations: influence of wheat roots and mercury addition. *Appl Environ Microbiol* **64:** 1210–1219.

Swofford, D.L. (1998) PAUP*. *Phylogenetic Inference Using Parsimony* (*and other methods). Version 4. Sunderland, MA, USA: Sinauer Associates.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25:** 4876–4882.

Venkatesan, M.M., Goldberg, M.B., Rose, D.J., Grotbeck, E.J., Burland, V., and Blattner, F.R. (2001) Complete DNA sequence and analysis of the large virulence plasmid of *Shigella flexneri*. *Infect Immun* **69:** 3271–3285.

Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., *et al.* (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304:** 66–74.

Vetriani, C., Chew, Y.S., Miller, S.M., Yagi, J., Coombs, J., Lutz, R.A., and Barkay, T. (2005) Mercury adaptation among bacteria from a deep-sea hydrothermal vent. *Appl Environ Microbiol* **71:** 220–226.

Weast, R.C. (1973) *Handbook of Chemistry and Physics*. Cleveland, OH: CRC Press.

Weber, J.H., Evans, R., Jones, S.H., and Hines, M.E. (1998) Conversion of mercury(II) into mercury(0), monomethylmercury cation, and dimethylmercury in saltmarsh sediment slurries. *Chemosphere* **36:** 1669–1687.

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