

First evidence of a mercury resistance mechanism in an anaerobic bacterium: impact on mercury sensitivity, accumulation and, methylation

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Mercury (Hg) is a persistent pollutant leading to environmental and health issues. While Hg resistance mechanisms are well characterized in aerobic microorganisms, they remain unidentified in anaerobes. Since some anaerobic bacteria are able to transform Hg(II) into methylmercury (MeHg), a potent neurotoxin, deciphering Hg resistance mechanisms is necessary to understand how anaerobes deal with Hg toxicity. Previous differential transcriptomic analysis on the anaerobic bacterium *Pseudodesulfovibrio hydrargyri* BerOc1 identified a cluster of genes overexpressed at 0.5 μM of Hg(II). They display sequence homologies with both efflux and metal resistant systems. This study aims to determine the role of this cluster in Hg resistance and methylation. Single BerOc1 mutant strain, deleted of the efflux system, and double mutant strain, deleted of both the efflux and metal resistant systems, were generated to conduct a comparative phenotypic analysis with the wild-type (wt). Sensitivity to Hg(II) and to MeHg was monitored by following bacterial growth, while MeHg production and intracellular Hg(II) concentrations were measured by GC-ICP-MS on cells exposed to different Hg(II) concentrations (from 0.0005 to 50 μM). Below 0.5 μM of Hg(II), no differences were observed for bacterial growth and intracellular Hg(II) concentration between wt and mutant strains. However, above 0.5 μM , sensitivity to Hg(II) increased for mutant strains with a higher impact for the double mutant. Therefore, 0.5 μM of Hg(II) was determined as the threshold concentration inducing resistance in the wt strain. Moreover, above 0.5 μM , single mutant strain accumulated 2 to 10 times more Hg(II), whereas double mutant accumulated 2 to 200 times less Hg(II) compared to wt depending on Hg(II) concentrations. Our results support a

two-level resistance mechanism: a first resistance (low level) induced by a periplasmic Hg-scavenging (metal resistant) and a second resistance (higher level) induced by the efflux system. Interestingly, MeHg production decreased in both mutant strains, even in the single mutant that showed higher intracellular Hg(II) contents. The accumulated Hg(II) was not available for methylation. This study highlights, for the first time, a mechanism of Hg resistance in anaerobic bacteria. This finding is a key step toward understanding the MeHg production and Hg(II) dissemination in environmental ecosystems.