Vinyl Chloride Reductase (*vcrA*) Identified on Extrachromosomal Circular Element in *Dehalococcoides mccartyi*

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Background/Objectives. Chlorinated solvents are present at almost 65% of U.S.A. DoD contaminated sites, with chlorinated volatile organic compounds, such as perchloroethene (PCE) and trichloroethene (TCE) confirmed at 36% of these sites. Bioaugmentation, using a mixed microbial culture, is an effective technology available for stimulating complete detoxification of groundwater contaminated with PCE or TCE. The KB-1 mixed microbial culture is well-suited for this purpose containing multiple strains of *Dehalococcoides mccartyi* capable of stepwise dechlorination of PCE to ethene.

D. mccartyi are hydrogen-utilizing, obligate organohalide-respiring bacteria. Their genomes contain two highly variable regions with multiple mechanisms for genetic recombination. Variable regions contain dozens of genes encoding reductive dehalogenases involved in organohalide respiration, including the vinyl chloride (VC) reductase gene (*vcrA).*
The VcrA enzyme is critical for effective clean-up of sites contaminated with chlorinated ethenes catalyzing the degradation of VC, a known human carcinogen, to non-toxic ethene. As such, the *vcrA* gene is commonly used as a biomarker during in situ bioremediation. Interestingly, there have been multiple reports of higher *vcrA* copy numbers than highly conserved, single-copy *D. mccartyi* 16S rRNA gene copies from field and laboratory samples. Sites at which PCE or TCE degradation results in the accumulation of VC have been a subject of debate in research with significant economic and environmental consequences.
We hypothesized that the high copy numbers of *vcrA* to 16S rRNA are attributed to processes involving horizontal gene transfer of *vcrA* by *D. mccartyi.* The purpose of our study was to explain these ratios and broaden our understanding of horizontal gene transfer, especially of *vcrA,* in *D. mccartyi*.

Approach/Activities. We used Illumina metagenomic sequencing and assembled complete genomes of *D. mccartyi* from KB-1 containing *vcrA*. We tracked the abundance of *vcrA* gene copies relative to 16S rRNA gene copies over three successive dechlorination cycles in triplicate laboratory cultures measured using quantitative PCR (qPCR). We designed multiple PCR reactions to determine the status of the *vcrA* containing genomic island (*vcrA*-GI).

Results/Lessons Learned. While assembling contigs, we found that the *vcrA*-GI was at twice the read depth compared to the rest of the genome. Furthermore, the metagenomic sequencing information linked the island to itself, suggesting the simultaneous occurrence of a circular intermediate and a chromosomal copy. In all three replicate culture bottles, we found that the ratio of *vcrA* to 16S copies was not constant: it varied from 1:1 to 2:1 in a somewhat cyclic fashion. We were able to amplify a single *vcrA*-GI from the genome and an extrachromosomal circular element using outward-facing PCR primers. We now know that the *vcrA*-GI can periodically exist as a circular, extrachromosomal element. Current and future research involves exploration of environmental pressures on *D. mccartyi*-containing cultures to induce horizontal gene transfer. The goal of this research is to provide insights on *D. mccartyi* during in situ bioremediation when high *vcrA* copy numbers are observed and VC has accumulated.