Project 1010098

# Genes for Uranium Bioremediation in the Anaerobic Sulfate-Reducing Bacteria

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**RESULTS TO DATE:** Genetics of Uranium Reduction by Sulfate-reducing Bacteria

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**Summary of project progress:**

**Objective A:** electron transfer components necessary for uranium reduction.

**Step 1:** Identify a nitrate-reducing Desulfovibrio strain that is genetically accessible. Desulfovibrio desulfuricans Essex 6 (ATCC 29577; NCIB 8307) has been tested for the introduction and maintenance of plasmid DNA by electrotransformation. Efficiencies of about 10-7 transformants/:g of DNA were obtained for stable plasmids. Transposon mutagenesis using the Tn5 delivery plasmid pRL1058 (designed for cyanobacteria by P. Wolk) occurred with an efficiency of about 10-8 mutants/ug of plasmid DNA. Examination of the transposon insertion Southern analysis revealed that in each of the six examined the transposon was in a DNA fragment of a different size. These results were consistent with the expectation that Tn5 would insert essentially randomly. Experiments with Desulfovibrio desulfuricans strain G20 have established that it is capable of U(VI) reduction. This is the strain in which we have constructed hydrogenase mutants and studied the phenotypes and we have shown that it is accessible by electrotransformation and conjugation. Transposon mutagenesis occurs at similar efficiencies as for Essex. The inability of this strain to use nitrate as a terminal electron acceptor means that mutations in electron flow to sulfate will be lethal unless we find an alternative growth mode. Attempts to use fumarate or other metals will be explored.

**Step 2:** Mutants of Desulfovibrio no longer able to reduce uranium. To date, about 100 independent transposon-containing derivatives of Essex have been collected for screening for U(VI) reduction, growth with nitrate as electron acceptor, and sensitivity to inhibition by molybdate. This rate of collection of mutations is painfully slow. We need to explore other transposons, other transposon constructs, and other means of getting transposition. Storage and manipulation of the mutations is also very time intensive. If we can generate numerous mutants, we can streamline the testing. Improving the efficiency of mutagenesis will be a high priority. We have explored the development of a colony assay for U(VI) reduction based on the loss of fluorescence of U(VI) when reduced to U(IV). This assay has not proven to be reliable since the non-fluorescing halos around the colonies are often quite small and the fluorescence of the solidified medium is not always uniform. We are still exploring a facile colony assay. A possibility that we are looking into (cost, availability, etc.) is spiking small liquid cultures with 233U, separating the U(IV) (uncharged) from the U(VI) present as an ion, and counting in a scintillation counter. We have used arsenazo III (2,2'[1,8-dihydroxy-3,6-disulfo-2,7-naphthalene-bis(azo)] dibenzenearsonic acid) to form a colored complex with U(VI) that absorbs at 652 nm (adapted from Yong et al., Anal. Chim. Acta 329:173-179, 1996) to estimate the kinetics of reduction of U(VI) by Desulfovibrio. A rate of about 0.015 to 0.03 mmol of U(VI) /hAmg whole-cell protein was observed for both strain G20 and Essex. This is about four fold less than the rates reported from Lovley's group (Lovley and Phillips, Environ. Sci. Technol. 58:850-856, 1992) but may be more rapid than the rates observed by Sayler and coworkers (Ganesh et al., Appl. Environ. Microbiol. 63:4385-4391, 1997). An operon encoding polypeptides with high homology to a NiFe hydrogenase and one potentially encoding an Fe hydrogenase have been disrupted in strain G20 by insertional duplication through the integration of a mutagenic plasmid. These

mutants were found to still be capable of U(VI) reduction when assayed by visual uraninite precipitation. More quantitative assays will reveal whether the reduction occurs with wild-type rates. In addition, we have access to the following hydrogenase mutants from Desulfovibrio fructosovorans: MR400, lacking a NiFe hydrogenase; SM4, lacking a NADP-reducing hydrogenase; DM4, lacking both the NiFe and NADP-reducing enzymes; and TM4, lacking the NiFe, NADP-reducing, and a recently discovered Fe-only hydrogenase. These assays should give a better picture of the involvement of hydrogenases in uranium reduction in Desulfovibrio. For another project, we have been attempting to confirm and study a mutant of G20 disrupted in cycA, the gene encoding the cytochrome c3. We have confirmed that such a mutant has been generated, but that it is quite unstable when the mutant cultures enter stationary phase, with suppressors rapidly overtaking the mutant. This mutant will be tested for uranium reduction. We have initiated the identification of the nitrate reductase of Essex to generate a mutant unable to grow with nitrate as terminal electron acceptor. This mutant will be tested for U(VI) reduction, although we anticipate that the rates will be wild type. However, a analysis of the regulation of the gene encoding this enzyme should lead us to global regulators responsible for controlling the expression of terminal electron acceptor pathways. We have designed primers from conserved regions of the gene and generated a PCR product that, when sequenced, has high identity with nitrate reductase genes. This amplicon is being used as a probe for isolation of the gene.

**Objective B:** Possible FNR-analog in the sulfate-reducing bacteria. Attempts to isolate FNR or FIKJ analogs from Desuflovibrio through the design of degenerate primers for amplification of portions of the genes has not been successful. In contrast, several amplicons have been generated for the genes encoding the regulators of two-component signal sequences. Since several global regulators fall into this class, we are attempting to obtain sufficient sequence information to indicate what metabolic pathways are affected by the regulators. Cloning and sequencing of two such amplicons has revealed that bona fide two-component regulators are present in Desulfovibrio.

**DELIVERABLES:** In preparation: Rapp-Giles, B. J., L. Casalot, R.S. English, J.A. Ringbauer, Jr., A. Dolla, and J.D. Wall. Cytochrome c3 mutants of Desulfovibrio desulfuricans. (for submission to J. Bact. or Appl. Environ. Microbiol. in July, 1999)

Payne, R.B., L. Casalot, J.A. Ringbauer, Jr., and J.D. Wall. Uranium reduction by hydrogenase mutants of Desulfovibrio.