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Genes for Uranium Bioremediation in the Anaerobic Sulfate-Reducing Bacteria

Wall, Judy D.
University of Missouri

RESULTS TO DATE: Genetics of Uranium Reduction by Sulfate-reducing Bacteria Judy D. Wall, PI Laurence Casalot, Postdoctoral Fellow, Nov. 98 - present Rayford Payne, Graduate Student Christopher L. Hemme, Graduate Student Biochemistry Department University of Missouri-Columbia Columbia, MO 65211

Summary of Progress (6/1/99-6/1/00): The objectives of the previous grant period were designed to explore the electron transport pathway employed by the sulfate-reducing bacteria (SRB) for the reduction of U(VI) to U(IV). More specifically experiments were designed to determine whether U(VI) reduction by members of the genus Desulfovibrio was mediated by a unique, dedicated reductase or occurred as a fortuitous reaction with a reductase naturally involved in alternative reduction processes. In addition, the regulation of the hierarchical expression of terminal electron acceptors (reductases) in the SRB was to be examined.

The first question to be resolved to achieve these objectives was to identify a Desulfovibrio strain that could reduce uranium, could use a non-sulfidogenic substrate for growth, and was genetically amenable. The acquisition of a Kinetic Phosphorescence Analyzer eliminated the restriction of mutant screens to a non-sulfidogenic growth mode. The D. desulfuricans strain G20, with which we have several years of experience, was shown to be capable of U(VI) reduction and mutants of potentially interesting electron transfer components have been constructed.

A mutation in cycA, the gene encoding cytochrome c3, was created in strain G20 (Rapp-Giles et al., 2000). Our progress with the study of that mutant is summarized below.

a) The cytochrome c3 gene, cycA, from D. desulfuricans G20 was isolated and sequenced. b) An internal SacII/PstI fragment was used to construct a mutagenic plasmid. c) The gene was disrupted by plasmid integration and confirmed by Southern hybridization. d) Northern analysis of the expression of the gene in the CycA mutant revealed a greater than 10 fold decrease in mRNA when compared with expression in the wild type strain. e) Tests of growth rate of the CycA mutant versus the wild type showed no differences when grown with lactate as carbon and reductant source and sulfate as electron acceptor. However, the mutant consistently was less efficient in cell production with these substrates, achieving only 80 to 85% of the growth of the wild type. f) Growth of the mutant with pyruvate as carbon and electron source appeared to be more dramatically affected. g) Suppressors that restored a wild-type copy of the gene were strongly selected when the cells were allowed to remain in stationary phase for extended periods between subcultures. h) Although mutants of cycA are viable, the mutants are impaired in their efficiency of substrate utilization.

When this mutation was assayed for uranium reduction, the rate was greatly decreased when compared with that of the parental strain (Table 1). However, reduction was not eliminated. We interpreted these results to mean that cytochrome c3 is involved in the Table 1. U(VI) reduction by D. desulfuricans wild-type G20 and various mutants*

Strain Phenotype Reductant Source Lactate Pyruvate Hydrogen WT(G20) 80 " 40 60 " 10 20 " 5 JAR3 HynA 90 " 30 100 " 40 25 " 10 JAR6 HynA 70 " 20 80 " 40 30 " 10 JAR8 HydA 70 " 30 130 " 40 35 " 20 I2 CycA 45 " 20 25 " 5 8 " 1

* Reduction rates are given as micromol/mg cell protein per h " indicates plus or minus

electron flow to U(VI) but that additional routes for delivering electrons to this metal are also present. The identification of these additional components will be a goal of the next phase of work.

Surprising results were obtained following an attempt to induce or derepress the machinery for U(VI) reduction by growing the cells in the presence of 1 mM uranyl acetate. G20 cells grown on lactate-sulfate medium amended with U(VI) reduced uranium at a slower rate than cells grown in the absence of this metal. When periplasmic extracts of these cells were prepared, Western analysis of the proteins revealed that the cytochrome c3 was absent. The Western analysis was performed on periplasmic extracts prepared as a high pH wash of 50-fold concentrated cells. Visible spectra of extracts of whole cells showed that the typical cytochrome c spectrum of the wild-type cells was no longer evident when the cells had been cultured with 1 mM uranium. This intriguing observation requires investigation and forms the basis of a major thrust of this proposal.

Preliminary experiments have eliminated the possibility that cytochrome c3 is lost from the periplasm as a result of damage occurring because of the precipitation of uraninite. Concentrated supernatants of cells grown with uranium show no detectable c3. In addition, the possibility that UO_2^{+} might compete for Fe^{2+} in regulation of the expression of iron-containing proteins, such as cytochromes, appears not to be the explanation. Western analysis of other periplasmic cytochromes suggest that the hexadecaheme (Hmc) and the newly discovered cytochrome cc3 are not detectably affected. Thus there is unlikely to be a problem with export, processing or loading of hemes. This phenomenon will continue to be analyzed and explored. Protein fusions have been created to follow the expression of cytochrome c3. They will be used to investigate the effects, if any, of exposure to uranium.

The identification of a hypothetical regulatory system for controlling expression of terminal electron acceptors in SRB is being pursued. The Essex strain grows either by sulfate reduction or by nitrate reduction. The nitrate reductase system appears to be repressed during growth on sulfate and to be increased in expression in the presence of nitrate. Degenerate primers to nitrate reductase from D. desulfuricans ATCC 27774 generated an internal fragment of the Essex nap gene that will be used for the isolation of the full gene and its promoter sequences. The regulation of this gene will be used to explore the possibility of a global transcriptional regulator for terminal electron acceptors.

DELIVERABLES: Publications and Presentations. Rapp-Giles, B. J., L. Casalot, R. S. English, J. A. Ringbauer, Jr., A. Dolla, and J. D. Wall. 2000. Cytochrome c3 mutants of Desulfovibrio desulfuricans. Appl. Environ. Microbiol. 66:671-677.

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