FINAL TECHNICAL REPORT
DE-FG02-05ER64124

Microbial Tc(VII) reduction is an attractive alternative strategy for bioremediation of technetium-contaminated subsurface environments. Traditional ex situ remediation processes (e.g., adsorption or ion exchange) are often limited by poor extraction efficiency, inhibition by competing ions and production of large volumes of produced waste. Microbial Tc(VII) reduction provides an attractive alternative in situ remediation strategy since the reduced end-product Tc(IV) precipitates as TcO_2, a highly insoluble hydrous oxide. Despite its potential benefits, the molecular mechanism of microbial Tc(VII) reduction remains poorly understood. The main goal of the proposed DOE-NABIR research project is to determine the molecular mechanism of microbial Tc(VII) reduction. Random mutagenesis studies in our lab have resulted in generation of a set of six Tc(VII) reduction-deficient mutants of *Shewanella oneidensis*. The anaerobic respiratory deficiencies of each Tc(VII) reduction-deficient mutant was determined by anaerobic growth on various combinations of three electron donors and 14 terminal electron acceptors. Results indicated that the electron transport pathways to Tc(VII), NO_3^-, Mn(III) and U(VI) share common structural or regulatory components. In addition, we have recently found that wild-type *Shewanella* are also able to reduce Tc(IV) as electron acceptor, producing Tc(III) as an end-product. The recent genome sequencing of a variety of technetium-reducing bacteria and the anticipated release of several additional genome sequences in the coming year, provides us with an unprecedented opportunity to determine the mechanism of microbial technetium reduction across species and genus lines.

Microbial metal reduction forms the basis of alternate bioremediation strategies for reductive precipitation and immobilization of toxic metals such as the radionuclide technetium [Tc(VII)]. A rapid mutant screening technique was developed to identify *Shewanella oneidensis* MR-1 respiratory mutants unable to reduce Tc(VII) as anaerobic electron acceptor. The Tcr mutant screening technique was based on the observation that wild-type *S. oneidensis* produced a black Tc(IV) precipitate on its colony surface during growth on Tc(VII)-amended agar, while colonies arising from mutagenized cells did not. Tcr mutants unable to produce the black precipitate were subsequently tested for anaerobic growth on an array of three electron donors and 13 alternate electron acceptors. The Tcr mutants displayed a broad spectrum of anaerobic growth deficiencies, including several that were unable to reduce Tc(VII) with hydrogen or lactate as electron donor, yet retained the ability to reduce Tc(VII) with formate. This study describes the development of a novel Tcr mutant screening technique and its application to identify the first set of Tcr mutants in a metal-reducing member of the genus *Shewanella*.

The putative Tcr mutants were subsequently grown either aerobically or anaerobically in minimal medium with fumarate as electron acceptor. After aerobic growth, Tcr mutants Tcr-9, Tcr-17 and Tcr-18 were unable to reduce Tc(VII) with either lactate or H_2. Tcr-9, however, displayed the ability to reduce Tc(VII) with formate as electron donor, while Tcr-17 and Tcr-18 did not. After anaerobic growth with fumarate

as electron acceptor, Tcr-17 was unable to reduce Tc(VII) with any electron donor. Tcr-18 was unable to reduce Tc(VII) with lactate or formate (yet displayed activity with H_2), while Tcr-9 reduced Tc(VII) with all three electron donors. These results indicate that Tc(VII) reduction in *S. oneidensis* MR-1 proceeds via electron transport pathways that are electron donor-specific and whose expression depends on the electron acceptor used for growth. Genetic complementation analysis is currently underway to identify the genes required for Tc(VII) reduction activity.

PUBLICATIONS RESULTING FROM PROJECT

Payne, A. and T. DiChristina. 2006. A rapid mutant screening technique for detection of technetium [Tc(VII)] reduction-deficient mutants of Shewanella oneidensis MR-1. FEMS Microbiology Letters, 259:282-287.

Dale, J., R. Wade and T. DiChristina. 2007. A conserved histidine in cytochrome *c* maturation permease CcmB of *Shewanella putrefaciens* is required for anaerobic growth below a threshold standard redox potential. *Journal of Bacteriology*, 189:1036-1043.

Burns, J., T. DiChristina, L. Wei and M. Urban. 2008. Affinity purification of tag-free *Shewanella oneidensis* cystathionine beta-lyase (MetC) using the Profinity eXact fusion-tag and Profinia protein purification systems. *Chromatography*, 128:26-30.

DiChristina, T., D. Bates, J. Burns, J. Dale and A. Payne. 2006. Microbial metal reduction by members of the genus Shewanella: novel strategies for anaerobic respiration. In L. Neretin (ed.), Biogeochemistry of Anoxic Marine Basins. Kluwer Publ. Co., Dordrecht, NL.

DiChristina, T., J. Fredrickson and J. Zachara. 2005. Enzymology of electron transport: Energy generation with geochemical consequences. Rev. Mineral. Geochem., 59:27-52.

INVITED TALKS RESULTING FROM PROJECT

DiChristina, T. 2008. Molecular mechanism of microbial metal respiration. Telluride Conference on Biogeochemical Processes of the Iron Cycle, Telluride, CO, 7/08.

DiChristina, T. 2008. Molecular mechanism of microbial metal respiration. 45th Annual Meeting of the Clay Minerals Society, New Orleans, LA, 4/08.

DiChristina T. 2007. Molecular mechanism of microbial metal respiration. Department of Microbiology, Cornell University, Ithaca, NY, 4/07.

DiChristina T. 2007. Molecular mechanism of microbial metal respiration. DOE-ERSP meeting, Washington, DC, 4/07.

DiChristina T. 2006. Molecular mechanism of microbial uranium and technetium reduction. American Chemical Society national Meeting. San Francisco, CA, 9/06.

DiChristina T. 2006. Molecular mechanism of microbial metal respiration. Gordon Research Conference on Environmental Bioinorganic Chemistry. Andover, NH, 6/06.

DiChristina T. 2006. Molecular mechanism of microbial metal respiration. 106th General Meeting, American Society for Microbiology. Orlando FL, 5/06.

DiChristina T. 2006. Molecular mechanism of microbial metal respiration. University of Texas – Pan American, Edinburg, TX, 5/06.

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DiChristina, T. 2005. Molecular mechanism of microbial metal respiration. Mineralogical Society of America, Short Course on Molecular Geomicrobiology, Berkeley, CA, 12/05.

DiChristina T. 2005. Molecular mechanism of microbial metal respiration. Allegheny College, Meadville, PA, 11/05.

DiChristina, T. 2005. New insights into the molecular mechanism of bacterial metal respiration. 15th Annual Goldschmidt International Meeting, Moscow, ID, 5/05.

DiChristina, T. 2005. Microbial Metal Respiration. Department of Geology and Environmental Sciences, Stanford University, Palo Alto, CA.

MEETING ABSTRACTS RESULTING FROM PROJECT

Payne, A. and T. DiChristina. 2006. Technetium reduction by Shewanella oneidensis MR-1. American Society for Microbiology National Meeting, Orlando, FL.

Payne, A. and T. DiChristina. 2005. Technetium reduction by Shewanella oneidensis MR-1. IUMS International Meeting, SanFrancisco, CA.

Payne, A. and T. DiChristina. 2005. Technetium reduction by Shewanella oneidensis MR-1. American Society for Microbiology National Meeting, Atlanta, GA