FINAL REPORT
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DOE NABIR AWARD #: DE-FG02-01ER63178
PROJECT TITLE: U(VI) Reduction-Specific Nucleic Acid and Antibody Probes
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Technetium Reduction. *Shewanella oneidensis* MR-1 respires anaerobically on a wide range of terminal electron acceptors, including oxidized forms of the radionuclides uranium [U(VI)] and technetium [Tc(VII)]. Microbial reduction of soluble Tc(VII) results in formation of Tc(IV) which precipitates as the highly insoluble hydrous oxide TcO_2, a Tc immobilization process that forms the basis of alternate remediation strategies. An alternate agar-based screening technique was developed for rapid identification of Tc(VII)-reduction deficient mutants in *S. oneidensis* MR-1. The mutant screen is based on the observation that wild-type MR-1 produces a black precipitate on its colony surface during Tc(VII) reduction that is absent on mutant colony surfaces. Chemically mutagenized cells of wild-type MR-1 were screened via the Tc(VII) agar assay, yielding six Tc(VII) reduction-deficient (Tcr) mutants. The anaerobic respiratory deficiencies of each Tcr mutant was determined by anaerobic growth on 14 terminal electron acceptors with either H_2, lactate or formate as electron donor. Results indicate that the electron transport pathways to Tc(VII), NO_3^-, Mn(III), U(VI) and $S_2O_3^-$ share common structural or regulatory components with H_2 as electron donor, but not with lactate or formate. These findings also indicate that anaerobic pathways in *S. oneidensis* MR-1 may be as highly branching at the head-end as they are at the terminal-end.

Uranium Reduction. In a previous study designed to identify genes required for U(VI) reduction, mutants of *S. putrefaciens* strain 200 were generated by ethyl methane sulfonate (EMS) mutagenesis and subsequently tested for anaerobic growth on nitrate, nitrite, sulfite, thilosulfate, Fe(III), Mn(IV), fumarate, or trimethylamine-N-oxide as electron acceptor. All Urr mutants were deficient in anaerobic growth on U(VI) and nitrite, including Urr mutant U14, which retained the ability to grow on all other electron acceptors. In the present study, a 13 kb wild-type DNA fragment from a *S. putrefaciens* gene clone bank restored U(VI) and nitrite reduction activity to U14. Nucleotide sequence analysis of the 13 kb fragment and complementation with PCR-amplified gene clusters indicated that the U14 mutation lies within the *ccmA* gene, an ABC transporter subunit of the cytochrome *c* maturation pathway. Additional growth analysis demonstrated that respiratory deficiency in U14 increases as the reduction potential of the electron acceptor decreases and respiratory ability decreases sharply when electron acceptors with reduction potentials less than nitrate are provided. These results support the hypothesis that one or more cytochromes is required for U(VI) reduction in *Shewanella putrefaciens* and that the CcmAB transporter is involved in redox homeostasis of the periplasm presumably by translocating an as yet unknown reductant that maintains heme iron in the ferrous state prior to apocytochrome ligation. In addition, these results suggest that the CcmAB transporter is only required for wild-type respiration activity below a threshold redox potential approximately equal to the reduction potential of amorphous Mn(IV).

Publications resulting from project:

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.

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 Publishing Co., Dordrecht, NL.

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.

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

Dale, J., R. Wade and T. DiChristina. 2004. Uranium reduction by *Shewanella oneidensis* MR-1 requires *nrfA*, a homolog of the *Escherichia coli* nitrite reductase structural gene (under review).

Moore, C. and T. DiChristina. 2004. Cytochrome c_3 deletion mutants of *Shewanella oneidensis* MR-1 retain the ability to respire all anaerobic electron acceptors (under review).

DiChristina, T., C. Moore and C. Haller. 2002. Dissimilatory Fe(III) and Mn(IV) reduction by *Shewanella putrefaciens* requires *ferE*, a *pulE* (*gspE*) homolog of Type II protein secretion. *J. Bacteriol.*, 184:142-151.

Haller, C. and T. DiChristina. 2002. Genetic approaches in bacteria with no natural genetic systems. *In* U. Streips and R. Yasbin (eds.), *Modern Microbial Genetics*, Second Edition. John Wiley and Sons, New York, pp. 581-602.

Moore, C. and T. DiChristina. 2002. Metal cycling. *In* G. Bitton (ed.), *The Encyclopedia of Environmental Microbiology*. John Wiley and Sons, New York, pp. 1902-1913.

Invited Talks resulting from Project:

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

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

DiChristina, T. 2004. Microbial Metal Respiration. Soil Science Society of America (SSSA) Annual Meeting, Seattle, WA.

DiChristina, T. 2004. New insights into the molecular mechanism of bacterial metal respiration. Clay Minerals Society (CMS) 41st Annual Meeting, Richland, WA.

DiChristina, T. 2004. Microbial Metal Respiration. Department of Biology, Montana State University, Bozeman, MN.

DiChristina, T. 2004. New insights into the molecular mechanism of bacterial metal respiration. Pacific Northwest National Laboratory (PNNL), Environmental Molecular Sciences Laboratory (EMSL) Annual Meeting on Structural Biology, Richland, WA.

DiChristina, T. 2004. New insights into the molecular mechanism of bacterial metal respiration. Department of Geology, University of Notre Dame, South Bend, IN.

DiChristina, T. 2004. New insights into the molecular mechanism of bacterial metal respiration. Department of Energy, Natural and Accelerated Bioremediation Research (NABIR) PI Meeting, Warrenton, VA.

Dale, J. and T. DiChristina. 2003. Uranium reduction by *Shewanella oneidensis* MR-1 shares nitrite respiration pathway components. American Chemical Society, Southeastern Regional Meeting, Atlanta, GA.

DiChristina, T. 2003. New insights into the molecular mechanism of bacterial metal respiration. Department of Energy, Grand Challenge Workshop "Electron transfer at the Microbe-Mineral Interface", Richland, WA.

DiChristina, T. 2003. New insights into the molecular mechanism of bacterial metal respiration. NSF-NATO, Advanced Research Workshop "Past and Present Water Column Anoxia", Crimea, Ukraine.

DiChristina, T. 2003. Microbial metal reduction. Gordon Research Conference on Applied and Environmental Microbiology, Connecticut College, New London, CT.

DiChristina, T. 2003. New insights into the molecular mechanism of bacterial metal respiration. Department of Microbiology, University of Georgia, Athens, GA.

Poster Presentations resulting from Project:

Payne, A. and T. DiChristina. 2004. Technetium reduction by *Shewanella oneidensis* MR-1. American Society for Microbiology National Meeting, New Orleans, LA.

Dale, J. and T. DiChristina. 2004. Uranium reduction by *Shewanella oneidensis* MR-1 shares nitrite respiration pathway components. American Society for Microbiology National Meeting, New Orleans, LA.

Adiga, M. and T. DiChristina. 2004. Dissimilatory Fe(III) and Mn(IV) reduction by *Shewanella oneidensis* requires *ferD*, a homolog of the *pulD (gspD)* Type II protein secretion gene. American Society for Microbiology National Meeting, New Orleans, LA.

Sankovich, K. and T. DiChristina. 2004. Mn(III) reduction by *Shewanella oneidensis* MR-1. American Society for Microbiology National Meeting, New Orleans, LA.

Payne, A. and T. DiChristina. 2003. Technetium reduction by *Shewanella oneidensis* MR-1. American Society for Microbiology National Meeting, Washington, DC.

Dale, J. and T. DiChristina. 2003. Uranium reduction by *Shewanella oneidensis* MR-1 shares nitrite respiration pathway components. American Society for Microbiology National Meeting, Washington, DC.

Graziano, S. and T. DiChristina. Dissimilatory Fe(III) and Mn(IV) reduction by *Shewanella oneidensis* requires *ferD*, a homolog of the *pulD (gspD)* Type II protein secretion gene. American Society for Microbiology National Meeting, Washington, DC.

Payne, A. and T. DiChristina. 2003. Technetium reduction by *Shewanella oneidensis* MR-1. American Society for Microbiology, Southeastern Branch Meeting, Athens, GA.

Dale, J. and T. DiChristina. 2003. Uranium reduction by *Shewanella oneidensis* MR-1 shares nitrite respiration pathway components. American Society for Microbiology, Southeastern Branch Meeting, Athens, GA.

Adiga, M. and T. DiChristina. Dissimilatory Fe(III) and Mn(IV) reduction by *Shewanella oneidensis* requires *ferD*, a homolog of the *pulD (gspD)* Type II protein secretion gene. American Society for Microbiology, Southeastern Branch Meeting, Athens, GA.

Sankovich, K. and T. DiChristina. 2003. Mn(III) reduction by *Shewanella oneidensis* MR-1. American Society for Microbiology, Southeastern Branch Meeting, Athens, GA.