

## Department of Energy Final Technical Report

Title: Defining How a Microbial Cell Senses and Responds to a Redox Active Environment

Grant Number: DOE Grant FG02\_02ER63427

PI: Kenneth Nealson, University of Southern California

### Summary of Accomplishments:

This grant was for four years, and the work was designed to look at the mechanisms of extracellular electron transfer by the dissimilatory iron reducing bacteria *Shewanella oneidensis* MR-1, and other closely related *Shewanella* strains and species.

During this work, we defined many of the basic physiological and biochemical properties of the *Shewanella* group, Much of which was summarized in review articles (refs. # 3, 7, & 8). We also finished and published the genome sequence of strain MR-1, the first of the shewanellae to have its genome sequenced (ref. #4).

Control at the transcriptional and translational level was studied in collaboration with colleagues at PNNL (refs. # 1, 2) and ANL (ref. # 6, 14, & 16).

We utilized synchrotron X-ray radiation to image both the bacteria and the metal oxide particles via a technique called STXM (ref. #5), synchrotron X-ray absorption (ref. #9), and X-ray microbeam analysis (ref. #10).

We purified several of the cytochromes involved with metal reduction (ref. #11), and improved gene annotation of the MR-1 genome (ref. # 12).

The conductive appendages (nanowires) of MR-1 were described and characterized. (ref. # 15).

Comparative genomics and biochemistry revealed that the pathway for the utilization of N-acetyl glucosamine in the various strains of *Shewanella* exhibited great variability, and had a number of previously unknown genes.

### Education:

During this time, several graduate students and postdocs were trained in the Nealson lab:

Postdocs: Andrea Belz  
Lea Cox  
Klaus Pecher  
Robert Rye  
Shunichi Ishii  
Radu Popa

#### Graduate Students:

Rachel Schelble  
Orianna Bretschger  
Lewis Hsu

In addition, several undergraduates were involved with this work each summer, and during the academic year.

#### Publications:

1. Thompson, D.K., A.S. Beliaev, C.S. Giometti, S.L. Tollaksen, T. Khare, D.P. Lies, K.H. Nealson, H. Lim, J. Yates III, C.C. Brandt, J.M. Tiedje, and J. Zhou. 2002. Transcriptional and proteomic analysis of a ferric uptake regulator (Fur) mutant of *Shewanella oneidensis*: Possible involvement of Fur in energy metabolism, transcriptional regulation, and oxidative stress. *Appl. Environ. Microbiol.* 68(2):881-892.
2. Beliaev, A.S., D.K. Thompson, T. Khare, H. Lim, C.C. Brandt, G. Li, A.E. Murray, J.F. Heidelberg, C.S. Giometti, J. Yates III, K.H. Nealson, J.M. Tiedje, and J. Zhou. 2002. Gene and protein expression profiles of *Shewanella oneidensis* during anaerobic growth with different electron acceptors. *OMICS: A Journal of Integrative Biology*, 6(1):
3. Nealson, K.H., A. Belz, and B. McKee. 2002. Breathing metals as a way of life: geobiology in action. *Ant. V. Leeuwenh.* 81:215-222.
4. Heidelberg, J. and others. 2002. Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis* *Nature Biotech.* 20, 1118 – 1123
5. Pecher, K., D. McCubbery, E. Kneidler, J. Rothe, J. Bargar, G. Meigs, L. Cox, K. Nealson and B. Tonner. 2003. Quantitative charge state analysis of manganese biominerals in aqueous suspension using Scanning Transmission X-ray Microscopy (STXM). *Geochim Cosmochim. Acta* 67:1089-1098
6. Giometti, C.S., T. Khare, S.L. Tollaksen, A. Tsapin, W. Zhu, J.R. Yates III, and K.H. Nealson. 2003. Analysis of the *Shewanella oneidensis* proteome by two-dimensional gel electrophoresis under non-denaturing conditions. *Proteomics* 3: 1-15.
7. Nealson, K.H., and J. Scott. 2003. Ecophysiology of the Genus *Shewanella*. In: Dworkin et al. eds. *The Prokaryotes: An evolving electronic resource for the microbiological community.* 3<sup>rd</sup> Ed. Release 3.7., Nov. 2003. Springer-Verlag, N.Y. <http://link.springer-ny.com/link/service/books/10125/>.
8. Nealson, K.H. and R. Rye. 2003. Evolution of Metabolism. Pp.41-61 in: *Treatise on Geochemistry, Volume 8.* Ed. W.H. Schlesinger, H.D. Holland, and K.K. Turekian. Elsevier Pergamon, Amsterdam.
9. Gilbert, B., B.H. Frazer, A. Belz, P. Conrad, K. Nealson, D. Haskel, J.C. Lang, G. Srajer, and G. DeStasio. 2003. Multiple scattering calculations of bonding and X-ray absorption spectroscopy of manganese oxides. *J. Phys. Chem. A* 107:2839-2847
10. Kemner, K.M., S.D. Kelly, B. Lal, J. Maser, E. J. O'Loughlin, D. Sholto-Douglas, Z. Cai, M. Schneegurt, C.F. Kulpa Jr., K.H. Nealson. 2004. Elemental and redox analysis of single bacterial cells by X-ray microbeam analysis. *Science* 306:686-687.

11. Meyer, T.E., A.I Tsapin, I. Vandenberghe, L. DeSmet, D. Frishman, K.H. Nealson, M.A. Cusanovich, and J.J. VanBeeumen. 2004. Identification of 42 possible cytochrome C genes in the *Shewanella oneidensis* genome and characterization of six soluble cytochromes. OMICS: J. Integr. Biol. 8:57-77
12. Kolker, E., A.F. Picone, M.Y. Galperin, M.F. Romine, R. Higdon, K.S. Makarova, N. Kolker, G.A. Anderson, X. Qiu, K.J. Auberry, G. Babnigg, A.S. Beliaev, P. Edlefsen, D.A. Elias, Y. Gorby, T. Holzman, J. Klappenback, K. T. Konstantinidis, M.L. Land, M.S. Lipton, L. McCue, M. Monroe, L. Pasa-Tolic, G. Pinchuk, S. Purvine, M. Serres, S. Tsapin, B.A. Zakrajsek, W. Zhu, J. Zhou, F.W. Larimer, C. Lawrence, M. Riley, F.R. Collart, J.R. Yates, III, R.D. Smith, C. Giometti, K. Nealson, J.K. Fredrickson, and J.M. Tiedje. 2005. Global profiling of *Shewanella oneidensis* MR-1: Expression of hypothetical genes and improved functional annotations. Proc. Nat. Acad. Sci. USA. 102:2099-2014.
13. Nealson, K.H. 2005. Hydrogen and energy flow as “sensed” by molecular genetics. Proc. Nat. Acad. Sci. USA 102: 3889-3890.
14. Beliaev, A.S., D.M.Stanek, J.A. Klappenbach, L. Wu, M.F. Romine, J.M. Tiedje, K.H. Nealson, J.K. Fredrickson, and J. Zhou. 2005. Global transcriptome analysis of *Shewanella oneidensis* MR-1 exposed to different terminal electron acceptors. J. Bacteriol. 187:7138-7145.
15. Gorby, Y., S. Yanina, J.S. McLean, K.M. Rosso, D. Moyles, A. Dohnalkova, T.J. Beveridge, I-S. Chang, B-H. Kim, K-S. Kim, D.E. Culley, S.B. Reed, M.F. Romine, D.A. Saffarini, E.A. Hill, L. Shi, D.A. Elias, D.W. Kennedy, G. Pinchuk, D. Watanabe, S. Ishii, B. Logan, K.H. Nealson, and J.K. Fredrickson. 2006. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. Proc. Nat. Acad. Sci. U.S.A. 103:11358-11363.
16. Gao, H., A. Obratzsova, N. Stewart, R. Popa, J.K. Fredrickson, J.M. Tiedje, K.H. Nealson, and J. Zhou. 2006. *Shewanella loihica* sp. Nov., isolated from iron-rich microbial mats in the Pacific Ocean. Int. J. Syst. Evol. Microbiol. 56: 1911-1916.
17. Yang, X., Rodionov, D., C. Li, O.N. Laikova, M.S. Gelfand, O.P. Zagnitko, M.F. Romine, A.Y. Obratzsova, K.H. Nealson, and A.L. Osterman. 2006. Comparative genomics and experimental characterization of N-acetylglucosamine utilization pathway of *Shewanella oneidensis*. J. Biol. Chem. 281(40):29872-29875.