Title: GENOME-FACILITATED ANALYSES OF GEOMICROBIAL PROCESSES
Award #: DE-FG02-04ER63882
Sponsor/Agency: Department of Energy
Project Period: 08/15/04-08/14/08

Final Technical Report:

This project had the goal(s) of understanding the mechanism(s) of extracellular electron transport (EET) in the microbe *Shewanella oneidensis* MR-1, and a number of other strains and species in the genus *Shewanella.*

The work was done primarily in the Nealson laboratory at the University of Southern California (USC), though there were extensive collaborations with a number of other investigators from the Pacific Northwest Laboratory (PNL), the Oakridge National Laboratory (ORNL), and the Burnham Institute in La Jolla.

The rationale for this work was based on the extensive advances that we had made as members of the "*Shewanella* Federation".

Accomplishments:

Advances in understanding of *Shewanella*

With more than 20 genomes sequenced, it was possible to continue our detailed studies of one strain (MR-1), and to add the comparative genomic analyses of the other strains of this genus. Thus, we divide this technical report into two sections: Focus 1) advances in the physiology (regulation), biochemistry and genomics of strain MR-1; and, Focus 2) understanding of general properties of the group *Shewanella* via genomic comparisons. In addition, we have a number of publications that represent major advances in technology and/or understanding that are in a "miscellaneous" section, Focus 3.

Focus 1) Understanding the strain *Shewanella oneidensis* MR-1. This bacterium, when isolated by my laboratory, was one of the first dissimilatory metal oxide reducing bacteria (DMRB) described, and has been a model system for the study of EET since that time. Significant advances made during the work funded by this proposal include:

Single cell analyses: Working with Dr. Ken Kemner of ANL, we successfully showed that the synchrotron X-ray microbeam facility could be used to characterize the redox state, and elemental composition of single cells of MR-1. This work resulted in a publication in Science (1).

Characterization of purified enzymes as c-type cytochromes in MR-1: Working with Dr. Terry Meyer from ASU, we purified and characterized 6 soluble cytochromes from MR-1, and identified 37 others in the genome of MR-1 (2). This was the first major purification of such cytochromes, and began a series of efforts that culminated last year in the successful crystallization of MtrF, one of the major proteins involved with EET.

Global transcriptome analysis of MR-1: Working with many members of the SF, we successfully analyzed the transcriptome of MR-1 grown with a variety of different electron acceptors. This work was the beginning of a monumental effort in gene-chip analysis of MR-1 (3,4).

Identification and characterization of conductive nanowires from MR-1: A group effort of many SF members resulted in the publication of a paper (5) in which conductive nanowires from MR-1 were characterized with regard to their content and their electrical conductivity. This has led to a major effort by many others to understand the physical nature of the conductivity and the role of these structures in EET (11,20,21).

Impact of surface charge on bacterial motility and attachment: Experiments begun during the funding period were focused on the role of surface charge of particles and electrodes on the motility and attachment of MR-1. This work was finished after the grant was terminated, but is rightfully part of the product of this proposal. The work was published two years ago in PNAS (16).

Nitrogen oxide reduction in MR-1: In collaboration with the Tiedje lab at MSU, it was determined that the nitrate reduction system in MR-1 (and most other shewanellae isolates) was an atypical system, and not a true denitrification (12).

Rate of electron transfer to solid substrates: In experiments with poised electrodes, it was possible, for the first time, to measure the rate of flow of electrons to a solid substrate (20). This work was repeated in several other labs, which obtained similar results, and is also consistent with estimates made by the Nealson lab, using solid metal oxides as substrates.

Focus 2: Comparative analyses of different *Shewanella* strains

N-acetyl Glucosamine Utilization by strains of *Shewanella*. In collaboration with the Osterman laboratory at the Burnham Institute, we studied in detail the genetics (genomics), biochemistry, and physiology of strains of *Shewanella* isolated from a wide variety of habitats. Almost none of them had a complete pathway for the degradation of NAG, the principal monomer of chitin, yet almost all were capable of NAG degradation and growth on NAG. Characterization of these strains revealed many previously unknown genes in NAG degradation, and greatly enlarged our knowledge of the genus *Shewanella* (7).

Sugar Utilization by strains of *Shewanella.* Continued collaboration with the Osterman lab focused on the utilization of various sugars by the shewanellae, and revealed many differences in the strategies used by the shewanellae as compared to their close relatives in the enteric bacteria. This resulted in a large publication describing the detailed genomic/biochemical properties of sugar utilization in the shewanellae (19).

Systems Biology and Comparative Genomics of the shewanellae: Several publications were put together describing the use of comparative genomics for analyses of the group *Shewanella,* and these were a logical culmination of our genomic-driven research (10,15,18).

Focus 3: Miscellaneous

Comparative metal reduction and impact on geobiology of *Shewanella* strains: *Shewanella* strains were compared with regard to secondary minerals produced during metal reduction, and it was concluded that the different strains of *Shewanella* can, under identical chemical and physical conditions, produce different mineral deposits, implying that the production of secondary products is biologically mediated, and not simply a matter of supply of reduced metal ions (13, 14, 17).

Uptake and use of DNA as a carbon and nitrogen source: In collaboration with PNL scientists, we described the distribution of abilities of various strains of *Shewanella* to take up and utilize DNA as the sole source of C and energy. This is a known, but rather rare trait of heterotrophic bacteria (9).

General Article on Environmental Genomics: Dr. Nealson had the opportunity of co-authoring an article with Dr. J. Craig Venter, discussing the "state of the art" of environmental metagenomics, and where it was taking us (8). This field, especially in marine science, has been led by Venter and the DOE.

Publications from this proposal (those in **Bold** are from high-impact journals (e.g., PNAS, Science, Nature**)**

1. **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.**
2. 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
3. **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.

4. 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.
5. **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.**
6. Gao, H., A. Obraztsova, 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.
7. Yang, X., Rodionov, D., C. Li, O.N. Laikova, M.S. Gelfand, O.P. Zagnitko, M.F. Romine, A.Y. Obraztsova, 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.
8. Nealson, K.H. and J.C. Venter. 2007. Metagenomics and the global ocean survey: what's in it for us, and why should we care. The ISME Journal 1:185-187.
9. Pinchuk, G.E., C. Ammons, D.E. Culley, S-M. W. Li, J.S. McLean, M.F. Romine, K.H. Nealson, J.K. Fredrickson, and A. Beliaev. 2008. Utilization of DNA as a sole source of phosphorus, carbon, and energy by *Shewanella* spp.: ecological and physiological implications for dissimilatory metal reduction. Appl. Environ. Microbiol. 74:1198-1208.
10. **Fredrickson, J.K., M.F. Romine, A.S. Beliaev, J.M. Auchtung, M.E. Driscoll, T.s. Gardner, K.H. Nealson, A.L. Osterman, G. Pinchuk, J.L. Reed, D.A. Rodionov, J.L.M. Rodrigues, D.A. Saffarini, M. H. Serres, A.M. Spormann, I.G. Zhulin, and J. M. Tiedje. 2008. Towards Environmental Systems Biology of *Shewanella*. Nature Rev. Microbiol. 6:592-603**
11. El-Naggar, M., Y. A. Gorby, W. Xia, and K.H. Nealson. 2008. The Molecular Density of States in Bacterial Nanowires., Biophys. J. 95:10-12.
12. Gao, H., Z.K. Yang, S. Barua, S. B. Reed, M.F. Romine, K.H. Nealson, J.K. Fredrickson, J.M. Tiedje, and J. Zhou. 2009. Reduction of nitrate in

Shewanella oneidensis depends on atypical NAP and NRF systems with NapB as a preferred electron transport protein from CymA to NapA. The ISME J. 3:966-976.

13. Salas, E.C., W.M. Berelson, D.E. Hammond, A.R. Kampf and K. H. Nealson. 2009. The impact of bacterial strain on the products of dissimilatory iron reduction. Geochim. Cosmochim. Acta. (in press: doi: 10.1016/j.gca.2009.10.039)
14. Salas, E.C., W.M. Berelson, D.E. Hammond, A.R. Kampf, and K. H. Nealson. 2009. The influence of carbon source on the products of dissimilatory iron reduction. Geomicrobiol. J. 26:451-462. `
15. **Konstantinidis, K., et al. 2009. Comparative systems biology across an evolutionary gradient within the Shewanella genus. Proc. Nat. Acad. Sci. USA 106: 15909-15914.**
16. **Harris, H.W., M.Y. El-Naggar, O. Bretschger, M.J. Ward, M. F. Romine, A.Y. Obraztsova, and K.H. Nealson. 2010. Electrokinesis is a microbial behavior that requires extracellular electron transport. Proc. Nat. Acad. Sci. 107:326-331**
17. Salas, E.C., W.M. Berelson, D.E. Hammond, A.R. Kampf, and K.H. Nealson. 2010. The impact of bacterial strain on the products of dissimilatory iron reduction. Geochim. Cosmochim. Acta. 74:574-583.
18. Karpinets, T.V., A.Y Obraztsova, Y. Wang, D.D. Schmoyer, G.H. Kora, B.H. Park, M.H. Serres, M.F. Romine, M.L. Land, T.B. Kothe, J.K. Fredrickson, K.H. Nealson, and E.C. Uberbacher 2010. Conserved synteny at the protein family level reveals genes underlying Shewanella species' cold tolerance and predicts their novel phenotypes. Funct. Integr. Genomics 10: 97 – 110. (DOI 10.1007/s10143-009-0142-y)
19. Rodionov, D., C. Yang, X. Li, I. Rodionova, Y. Wang, A.Y. Obraztsova, O. P. Zagnitko, R. Overbeek, M. F. Romine, S. Reed, J.K. Fredrickson, K.H. Nealson, A.L. Osterman. 2010. Genomic encyclopedia of sugar utilization pathways in the Shewanella genus. BMC Genomics 2010, 11:494
20. McLean, J.S., G. Wanger, Y.A. Gorby, M. Wainstein, J. McQuaid, Shun'ichi Ishii, O. Bretschger, H. Beyanal, K.H. Nealson. 2010. Quantification of electron transfer rats to a solid phase electron acceptor through the stages of biofilm formation from single cells to multicellular communities. Env. Sci. Technol. 44:2721-2717.
21. **El-Naggar, M. , G. Wanger, K.M. Leung, T.D. Yuzvinsky, G. Southam, J. Yang, W.M. Lau, K.H. Nealson, and Y.A. Gorby. 2010. Electrical Transport Along Bacterial Nanowires from *Shewanella oneidensis* MR-1 Proc. Nat. Acad. Sci. USA 107:18127-18131.**

Students and Postdocs Trained during this work:

Graduate Students doing part or all of their Ph.D. work during this funded research:

1. Orianna Bretschger: Ph.D. Engineering -- USC* (2010)
2. John McCrow, Ph.D. Computational Biology – USC (2010)
3. Mike Waters, Ph.D. Computational Biology, USC (2009)
4. Everett Salas, Ph.D. Earth Sciences, USC** (2009)
5. Laurie Barge, Ph.D. Earth Sciences, USC* (2009)
6. Beverly Flood, Ph.D. Earth Sciences, USC* (2010)
7. Jeff McLean, Ph.D. in progress, Earth Sciences, USC
8. H. Wayne Harris, Ph.D. in progress, Biology, USC

Postdoctoral Associates:

1. Jinjun Kan, now at the Stroud Water Institute
2. Greg Wanger, now at the J.C. Venter Institute
3. Jason He, now at the Univ. of Wisconsin
4. Moh El-Naggar, now at USC (dept. of Physics)

Undergraduate Trainees:

We had, on the average ~ 5 undergrads per year in the laboratory, many of whom did undergraduate honors research, and participated in the poster competition at the end of the year. These students were paid in part, or completely by funds from the DOE grant.