Final Technical Report:

Award #: DE-FG02-07ER63882
Title: Genome-Based Studies of *Shewanella* Ecophysiology
DOERN
Project Period: 06/01/2009 – 05/31/2010
PI: Kenneth H. Nealson, Wrigley Professor of Environmental Sciences, USC

Summary Statement:

This project had as its goals the understanding of the ecophysiology of the genus *Shewanella* using various genomics approaches. As opposed to other programs involving *Shewanella*, this one branched out into the various areas in which *Shewanella* cells are active, and included both basic and applied studies. All of the work was, to some extent, related to the ability of the bacteria to accomplish electron exchange between the cell and solid state electron acceptors and/or electron donors, a process we call Extracellular Electron Transport, or **EET.** The major accomplishments related to several different areas:

Basic Science Studies:

1. Genetics and genomics of nitrate reduction, resulting in elucidation of atypical nitrate reduction systems in *Shewanella oneidensis* (MR-1)[2].
2. Influence of bacterial strain and growth conditions on iron reduction, showing that rates of reduction, extents of reduction, and the formation of secondary minerals were different for different strains of *Shewanella* [3,4,9].
3. Comparative genomics as a tool for comparing metabolic capacities of different *Shewanella* strains, and for predicting growth and metabolism [6,10,15]. In these studies, collaboration with ORNL, PNNL, and
4. Basic studies of electron transport in strain MR-1, both to poised electrodes, and via conductive nanowires [12,13]. This included the first accurate measurements of electrical energy generation by a single cell during electrode growth [12], and the demonstration of electrical conductivity along the length of bacterial nanowires [13].
5. Impact of surface charge and electron flow on cell movement, cell attachment, cell growth, and biofilm formation [7.18]. The demonstration that interaction with solid state electron acceptors resulted in increased motility [7] led to the description of a phenomenon called electrokinesis. The importance of this for biofilm formation and for electron flow was hypothesized by Nealson & Finkel [18], and is now under study in several laboratories.

Applications:

1. **Corrosion**: Electron flow is often part of the corrosive process, and several studies were done in concert with this proposal with regard to the ability of EET-capable bacteria to enhance, inhibit, or detect corrosion. These included using EET-capable bacteria to detect corrosion in its earliest stages [5], to use corrosion-causing bacteria for the study of the microbe/mineral interface during corrosion [1], and to study the groups of microbes involved with corrosion of natural systems [19].
2. **Bioenergy and microbial fuel cells:** The production of electricity by *Shewanella* was shown early in this program (several years ago) to be dependent on the genes for extracellular electron transport (EET), and applied work involved the testing of various strains and conditions for the optimization of current production by the shewanellae [11,14,16].
3. **Identification of shewanellae strains:** Based on similarities seen in genomic comparisons, a rapid method was employed for distinguishing between shewanellae strains [17].

Interactions with other laboratories:

This grant was an extension of a grant involving the so-called "Shewanella Federation", and as such, a number of our publications were joint with other members of this group. The groups included:

1. Pacific Northwest Laboratories – PNNL, Hanford, WA
2. Oak Ridge National Labs – ORNL, Oak Ridge, TN
3. Michigan State University, Tiedje Lab, East Lansing, MI
4. University of Oklahoma, Zhou lab,
5. Naval Research Laboratory, Biffinger, Washington DC
6. Burnham Medical Research Institute, San Diego, CA
7. J. Craig Venter Institute, San Diego, CA

Education:

Graduate Students:

Michael Waters, Ph.D. – now at NIST, Washington D.C.
Lewis Hsu, Ph.D. – now at NRL, San Diego
Howard Harris, Ph.D. – Postdoc at Universite de Pau, France
Everett Salas, Ph.D. – Scientist at Chevron, Sacramento, CA
McLean, Jeffrey, Ph.D. – Scientist at J. Craig Venter Institute, San Diego, CA
McCrow, John, Ph.D. – Scientist at J. Craig Venter Institute, San Diego, CA

Postdocs:

Mohamed El-Naggar – Professor of Physics, USC
Jinjun Kan – Senior Researcher at

Undergraduates:

During this year, we had three undergraduate researchers in the lab, each working on an aspect of this work, and each involved with an undergraduate honors research project.

Publications produced as a result of this funding.

1. Waters, M.S., M.Y. El-Naggar, L. Hsu, C.A. Sturm, A. Luttge, F.E. Udwadia, D.G. Cvitkovitch, S.D. Goodman, and K.H. Nealson. 2009. Simultaneous interferometric measurement of corrosive or demineralizing bacteria and their mineral interfaces. Appl. Environ. Microbiol. 75:1445-1449.
2. 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.
3. 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)
4. 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 [7,18].
5. Waters, M.S., E.C. Salas, S.D. Goodman, F.E. Udwadia, and K.H. Nealson. 2009. Early detection of oxidized surfaces using *Shewanella oneidensis* MR-1 as a tool. Biofouling. 25:163-172.

6. Konstantinidis, K., et al. 2009. Comparative systems biology across an evolutionary gradient within the Shewanella genus. **Proc. Nat. Acad. Sci. USA** 106: 15909-15914.
7. 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
8. Nealson, K.H. 2010. Sediment reactions defy dogma. **Nature** 463:1033-1034.
9. 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.
10. Karpinets, T.V., A.Y Obraztsova, Y. Wang, D.D. Schmoyer, G.H. Kora, B.H. Park, M.H. Serres, M.F. Ropmine, 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)
11. Bretschger, O., A.C.M. Cheung, F. Mansfeld, and K.H. Nealson. 2010. Comparative microbial fuel cell evaluations of *Shewanella* spp. Electroanalysis 22: 883-894.
12. 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 rates to a solid phase electron acceptor through the stages of biofilm formation from single cells to multicellular communities. Env. Sci. Technol. 44:2721-2717.
13. 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.
14. Biffinger, J.C., L.A. Fitzgerald, R. Ray, B.J. Little, S.E. Lizewski, E.R. Petersen, B.R. Ringeisen, W.C. Sanders, P.E. Sheehan, J.J. Pietron, J.W. Baldwin, L.J. Nadeau, G.R. Johnson, M. Ribbens, S.E. Finkel, K.H. Nealson. 2010. The utility of *Shewanella japonica* for microbial fuel cells. Bioresource Technol. 102:290-297.
15. 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
16. Kan, J., L. Hsu, A.C.M. Cheung, M. Pirbazari, and K.H. Nealson. 2011. Current production by bacterial communities in microbial fuel cells enriched from wastewater sludge with different electron donors. Env. Sci. Technol. 45: 1139-1146.
17. Kan, J. B. Flood, J.P. McCrow, J.S. Kim, L. Tan, and K.H. Nealson. 2011. A rapid fingerprinting approach to distinguish between closely related strains of *Shewanella.* J. Microbiol. Methods. 86: 62-68.
18. Nealson, K.H. and S.E. Finkel. 2011. Electron flow and biofilms. MRS Bull. 36:380-384.
19. Kan, J., P. Chellamuthu, A. Obraztsova, J.E. Moore, and K.H. Nealson. 2011. Diverse bacterial groups are associated with corrosive lesions at a Granite Mountain Record Vault (GMRV). J. Appl. Microbiol. 111: 329-337.

Patents and Inventions:
No patents or inventions were created during this funded work.

Protected Personally Identifiable Information: no PPII is included