Final Technical Report of Institute for Environmental Genomics of University of Oklahoma (DE-FG02-07ER64383)

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As a part of the *Shewanella* Federation project, we have used integrated genomic, proteomic and computational technologies to study various aspects of energy metabolism of two *Shewanella* strains from a systems-level perspective. Besides, a patent was filed for a mutant of *Shewanella loihica* PV-4 strain capable of accumulating protoporphyrin IX under U.S. Serial No. 14/349,171.

1. Characterization of nitrate reduction subsystems in *Shewanella*

The nitrate respiration subsystems are highly diverse among *Shewanella* species. The bioinformatics analyses demonstrated that there are three types of nitrate reduction systems in *Shewanella* genomes. The model systems proposed for experimental investigation include:

(i) *S. oneidensis* MR-1 with only the CymA-dependent nitrate reductase and the NapGH ubiquinol oxidase;

(ii) *S. putrefaciens* W3-18-1 with both the CymA- and NapC-dependent nitrate reductases, and the NapGH ubiquinol oxidase

(iii) *S. baltica* OS185 with both the CymA- and NapC-dependent nitrate reductases but without the NapGH ubiquinol oxidase.

S. oneidensis MR-1 serves as a model for studying anaerobic respiration and electron transport-linked metal reduction. In the genome of *S. oneidensis*, a *napDAGHB* gene cluster encoding periplasmic nitrate reductase (NapA of the NAP system) and accessory proteins and an *nrfA* gene encoding periplasmic nitrite reductase (NrfA of the NRF system) have been identified. However, these two systems appear to be atypical because the genome lacks both *napC* and *nrfH*, which are essential for reduction of nitrate to nitrite and nitrite to ammonium in most bacteria containing these two systems, respectively. In this study, we demonstrated that reduction of nitrate to ammonium in *S. oneidensis* is carried out by these atypical systems in a two-step manner. Unexpectedly, the *napB* mutant exhibited a higher maximum cell density than the wild-type while the *napA* mutant was defective completely in growth on nitrate. Although

reduction of nitrate to ammonium in the *napB* mutant is also conducted by NAP and NRF systems, nitrite, the intermediate of the reduction, was not detected through the entire reduction. Further investigation suggests that NapB may be the preferred electron acceptor from a membrane-bound protein which delivers electrons from menaquinol pool to a number of terminal reductases. In an attempt of searching for this membrane-bound protein, both microarrays and mutational analysis have been taken. The results suggest that CymA is likely to be functional replacement of both NapC and NrfH in the nitrate reduction and a model is proposed.

We have monitored the nitrate reduction in W3-18-1 and a closely related strain CN32, and the transient nitrite accumulation had been observed in both strains fed with 2mM of nitrate (lactate as electron donor), which suggests that the above-mentioned two-step manner of nitrate reduction may be common among *Shewanella* species. Our results suggest that the two-step manner of nitrate reduction found in MR-1 may be common among *Shewanella* species. Deletion of the *nap1* (*napDAGHB*) or *nap2* (*napDABC*) operon did not significantly affect cell growth, but the double mutant could not grow on nitrate, suggesting that the two *nap* operons are functionally redundant. In addition, the in-frame of *cymA* and *napC* of W3-18-1 deletion mutants did not show severe growth inhibition on nitrate, though deletion of *cymA* resulted in the loss of nitrate and nitrite reduction and growth in MR-1. Furthermore, the *cymA* deletion mutant showed little growth on nitrite in contrast to the *napC* deletion mutant, indicating that CymA was involved in nitrite reduction in both W3-18-1 and MR-1. The *cymA* gene from W3-18-1 complements the MR-1 *cymA* in-frame deletion mutant and allows reduction of ferric ions, nitrate, and nitrite when expressed *in trans*. The *napC* gene from W3-18-1 can also complement the MR-1 *cymA* deletion mutant and allows ferric iron reduction but it failed to allow nitrite reduction. These results support the hypothesis that the NapC-dependent and CymA-dependent periplasmic nitrate reduction systems allow an efficient dissimilatory reduction of nitrate and nitrite. Deletion of *narP* and *narQ* resulted in the growth inhibition on nitrate, suggesting that nitrate reduction is also regulated by the NarQP two- component system in W3-18-1. Our competition assays showed that W3-18-1 had a competitive advantage over MR-1 when grown together on nitrate.

2. Characterization of *c*-type cytochrome genes

S. oneidensis has a large number (41) of c-type cytochromes compared to best-studied *Escherichia coli*, which hosts only 5~7 c-type cytochromes. The enormous number of *c*-type

cytochromes has been linked to its diverse respiratory capability. A comparison among sequenced *Shewanella* revealed that twelve and three out of these 41 genes are present in all and just one less of *Shewanella* species, respectively, representing the core of c-type cytochrome genes (~36.6% in *S. oneidensis*) across the genus. While the analogues to 23 of the *S. oneidensis* genes are identified in at least one of other genomes, three appear in the *S. oneidensis* genome only. An attempt to delete all of c-type cytochrome genes was made and 36 mutants were obtained with either Fusion PCR or cre-lox approach. Among them, only five or so genes were found to have global effects on the bacterial respiration. To distinguish subtle impacts of these genes on the physiology of *S. oneidensis*, a competition study was used. Cytochrome mutants were equally mixed were grown together with different substrates under different condition, and the dynamics of their relative abundance was monitored by RT-PCR. Those competition assays revealed that SO0610, SO1777, SO2361, SO2363, and SO4360 were important under aerobic growth conditions, and that more *c*-type cytochomes showed significant impacts on anaerobiosis than aerobiosis, and CymA may be important for anaerobic growth, which is consistent with previous findings with individual characterization in general. Surprisingly, the *petC* deletion appeared not only to be important during aerobiosis, but also ananerobiosis. In addition, our results also showed that OS4666 might be important for pellicle biofilm formation.

3. Metal reduction in *S. putrefaciens* W3-18-1

S. putrefaciens W3-18-1 differs from *S. oneidensis* MR-1 in the metal reduction gene cluster significantly. *S. putrefaciens* W3-18-1 lacks orthologues for the secondary metal reductase and accessory proteins (MtrFED) of *S. oneidensis* MR-1. Sputw3181_2446 encodes a decaheme *c*-cytochrome, orthologous to the outer membrane primary metal reductase OmcB of MR-1 (60% similarity) while another reductase similar to OmcA in MR-1 was also found in W3-18-1. Sputw3181_2445 encodes an 11-heme *c*-type cytochrome OmcE, which only shares 40% similarity with the decaheme cytochrome OmcA. Single and double in-frame deletion mutants of *omcB* and *omcE* were generated for functional characterization of *omcE* and metal reduction in W3-18-1. Reduction of solid-phase Fe(III) and soluble Fe(III) in *S. putrefaciens* W3-18-1 was mainly dependent on OmcB under anaerobic conditions (with 50 mM lactate as electron donors and Fe_2O_3, alpha FeO(OH), beta FeO(OH) and ferric citrate as electron acceptors. W3-18-1 catalyzed a more rapid reduction of alpha FeO(OH) as compared to MR-1, suggesting that other genes may be involved in Fe(III) reduction in W3-18-1. As previously observed in MR-1, the

deletion of both OmcE and OmcB led to a severe deficiency in reduction of solid-phase Fe(III) in W3-18-1 and and an even greater deficiency in the reduction of soluble iron. The *omcB* and *omcE* genes of W3-18-1 have been expressed with the pBAD vector in *E. coli*. Heme staining assays also demonstrated that the disappearance of specific protein bands in the SDS-PAGE gels were consistent with *omcB* and *omcE* deletion in three mutant samples. These results suggest that *omcE* and *omcB* are actually expressed as cytochrome proteins and could play a central role in metal reduction in *S. putrefaciens* W3-18-1. Besides, the discovery of PSTI restriction enzyme system in W3-18-1 greatly improved mutagenensis efficiency.

4. Generation and validation of a *Shewanella oneidensis* MR-1 clone set for protein expression and phage display

An ORF clone set for *S. oneidensis* was created using the lambda recombinase system. ORFs within entry vectors in this system can be readily transferred into multiple destination vectors, making the clone set a useful resource for research groups studying this microorganism. Although the Gateway system offers multiple destination vectors for a variety of analyses, we developed a series of destination plasmids to better suit the needs of this study. To establish that the *S. oneidensis* clone set could be used for protein expression and functional studies, three sets of ORFs were examined for expression of His-tag proteins, expression of His/GST-tag proteins, or for effective display on phage. A total of 21 out of 30 (70%) predicted two-component transcriptional regulators from *S. oneidensis* were successfully expressed in the His-tag format. The use of the *S. oneidensis* clone set for functional studies was tested using a phage display system. The method involves the fusion of peptides or proteins to a coat protein of a bacteriophage. This results in display of the fused protein on the exterior of the phage, while the DNA encoding the fusion resides within the virion. The physical linkage between the displayed protein and the DNA encoding it allows screening of vast numbers of proteins for ligand-binding properties. With this technology, a phage clone encoding thioredoxin TrxA was isolated from a sub-library consisting of 80 clones. It is evident that the *S. oneidensis* clone set can be used for expression of functional *S. oneidensis* proteins in *E. coli* using the appropriate destination vectors. Destination vectors are available for a number of protein expression formats as well as for functional studies such as phage display and yeast two-hybrid assays.

5. Characterization of ArcA

In *Escherichia coli*, metabolic transitions between aerobic and anaerobic growth states occur when cells enter an oxygen-limited condition. Many of these metabolic transitions are controlled at the transcriptional level by the activities of the global regulatory proteins ArcA (aerobic respiration control) and Fnr (fumarate nitrate regulator). A homolog of ArcA (81% amino acid sequence identity) was identified in *S. oneidensis* MR-1, and *arcA* mutants with MR-1 as the parental strain were generated. Phenotype characterization showed the *arcA* deletion mutant grew slower than the wild-type and was hypersensitive to H_2O_2 stress. Microarray analysis indicated that S. *oneidensis* ArcA regulates a large number of different genes from that in *E. coli* although they do have overlapping regulatory functions on a small set of genes. The *S. oneidensis arcA* gene was also cloned and expressed in *E. coli*. The ArcA proteins from the wild-type and a point mutant strains (D54N) were purified and their DNA binding properties were analyzed by electrophoretic motility shift (EMS) and DNase I footprinting assays. The results indicate that phosphorylated ArcA proteins bind to a DNA site similar in sequence to the *E. coli* ArcA binding site. The common feature of the binding sites is the presence of a conserved 15 base pair motif that contains 2-3 mismatches when compared to the *E. coli* ArcA-P consensus binding motif. Genome scale computational predictions of binding sites were also performed and 331 putative ArcA regulatory targets were identified. Although the computational screening is in need of refinement, the results suggest the *S. oneidensis* and *E. coli* ArcA-P proteins may differ significantly in terms of the regulation of energy metabolism/respiration. Therefore, the regulation of aerobic/anaerobic respiration may be more complex than it was expected in *S. oneidensis*. We have also generated arcA mutants for W3-18-1, and proteomic studies were done at PNNL.

6. A high-throughput percentage-of-binding strategy to measure binding energies in DNA-protein interactions

Based on results of studies on ArcA of S. oneidensis, we developed a high-throughput approach to measure binding energies in DNA-protein interactions, which enables a more precise prediction for DNA-binding sites in genomes. With this approach, the importance of each position within the ArcA-P binding site was quantitatively established by characterizing the interaction between *Shewanella* ArcA-P and a series of mutant promoter DNAs, whereby each position in the binding site was systematically mutated to all possible single nucleotide changes. The results of the fine mapping were used to create a position-specific energy matrix (PEM) that

was used for a genome-scale prediction of 45 ArcA-P sites in *Shewanella*. A further examination suggests that this prediction is >81% consistency with *in vivo* gene regulation according to microarray studies and >92% (13/14) accuracy in comparison with published *in vitro* gel shift validation binding assays. In addition, this study predicted 27 ArcA-P sites for 15 published *E. coli* ArcA-P footprinted DNAs, and 24 of them were found exactly within the footprinting protected regions and the other three sites fall into the regions that were not examined by footprinting assays. This is the first report showing that footprinting protected regions can be effectively predicted by starting from a single known transcription factor binding site. Finally, the predicted *H. influenzae* ArcA-P sites correlate well with in vivo regulation determined by a microarray analysis in that the eight predicted binding sites with the most favorable ΔΔG scores all exhibit ArcA dependent gene regulation. The one-step percentage-of-binding strategy described in this study provides a rapid approach to examine binding energy in DNA-protein interactions via systematic mutation of the DNA binding site. In addition, the application of percentage-of-binding strategy to microarray-based DNA-protein interactions could result in a low cost and high throughput genome-scale site-discovery approach for many other transcription factors.

7. Genetic footprinting analysis on *Shewanella oneidensis* MR-1

The genetic footprinting approach combines the power of transposon mutagenesis and microarray analyses and it is an important tool to identify the conditionally essential genes and gene functions at a high-throughput manner in bacteria. We have already modified two widely used transposon delivery vectors, pBSL180 (*mini-Tn10*) and pFAC (*mariner*) by introducing an outwardly oriented T7 promoter into the transposon for this purpose.

a. The mini-Tn10 transposon delivery vector pBSL180

The pBSL180 encoded a conditionally regulated Tn*10* transposase with relaxed sequence specificity, which may lead to the randomly distributed insertions of transposon around the bacterial chromosome. The pilot experiments have been conducted on MR-1 and a few metal reduction relevant genes such as SO3669 were found. Since there are too many false positive results in the T7 polymerase mediated in vitro reverse transcription method, we have designed several other ways to identify the flanking regions of transposon.

b. The mariner transposon delivery vector pFAC and pMiniHimar RB1

The *mariner* transposaese recognizes only the dinucleotide TA for insertion, and therefore the transposon could potentially be inserted into each single gene around genome, except for the essential genes. We have tested the mariner delivery vector pFAC (Wong and Mekalanos, 2000) and pMinihimar RB1 (Bouhenni *et al*., 2005) in MR-1 and W3-18-1. We have also constructed transposon mutant library of W3-18-1 by using pMiniHimar RB1. This library could be subjected to different treatment to identify the conditionally essential genes.

8. Protoporphyrin-IX (PPIX) accumulating mutant of *Shewanella loihica* PV-4

In an effort to screen for temperature sensitive mutants of *Shewanella loihica* PV-4 from a mariner based transoposon mutant library, a few mutants were found to exhibit dark red color. Identification of transposon insertion revealed an interrupted hemH gene in PV-4. The accumulated heme synthesis intermediate, as tested by mass spectrum, fluorescence, UV-vis, FTIR, is protoporphyrin IX. This mutant and its capability to accumulate PPIX was filed for patent under U.S. Serial No. 14/349,171.

Recent Publications

A. FY2010-2013

1. Y Yang, J Chen, D Qiu, J Zhou (2013), Roles of UndA and MtrC of *Shewanella putrefaciens* W3-18-1 in iron reduction, BMC microbiology 13 (1), 267
2. D Qiu, H Wei, Q Tu, Y Yang, M Xie, J Chen, MH Pinkerton, Y Liang, Z He, J Zhou (2013) Combined genomics and experimental analyses of respiratory characteristics of *Shewanella putrefaciens* W3-18-1, Applied and environmental microbiology 79 (17), 5250-5257
3. Y. Yang, L. A. McCue, A. B. Parsons, S. Feng and J. Zhou (2010), The tricarboxylic acid cycle in *Shewanella oneidensis* is independent of Fur and RyhB control. BMC Microbiology 10:264
4. H. Gao, S. Barua, Y. Liang, L. Wu, Y. Dong, S. Reed, J. Chen, D. Culley, D. Kennedy, Y. Yang, Z. He, K. Nealson, J. Fredrickson, J. Tiedje, M. Romine, J. Zhou (2010), Impacts of *Shewanella oneidensis* c-type cytochromes on aerobic and anaerobic respiration, Microbial Biotechnology 3(4): 455-466
5. Y. Yang, D. P. Harris, F. Luo, W. Xiong, M. Joachimiak, L. Wu, P. Dehal, J. Jacobsen, Z. Yang, A. V. Palumbo, A. P. Arkin and J. Zhou (2009), Snapshot of Iron Response in *Shewanella oneidensis* by Gene Network Reconstruction, BMC Genomics 10:131

B. FY2009

1. Gao, H., Z.K Yang, S. Barua, S. B. Reed, M. F. Romine, K. H. Nealson, J. K. Fredrickson, J. M. Tiedje, and **J.Z. 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(8):966-76. Yang Y. , D. P. Harris, F. Luo, W. Xiong, M. Joachimiak, L. Wu, P. Dehal. J.Jacobsen, Z. Yang, A. V. Palumbo, A. P. Arkin, and **J.Z. Zhou**, 2009. Snapshot of Iron Response in *Shewanella oneidensis* by Gene Network Reconstruction. BMC Genomics, 10:131.

2. Yang, Y.F., L. A. McCue, A. B. Parsons, S. Feng, and **J.Z. Zhou**, 2009. Tricarboxylic compound utilization in *Shewanella oneidensis* is independent of Fur and RyhB control. Microbiology, in revision.

C. FY2007-2008

1. Gao, Haichun, Donna Pattison, Tingfen Yan, Dawn M. Klingeman, Xiaohu Wang, Joseph Petrosino, Lisa Hemphill, Xiufeng Wan, Adam B. Leaphart, George Weinstock, Timothy Palzkill, and Jizhong Zhou. 2008. Generation and Validation of a Shewanella oneidensis MR-1 Clone Set for Protein Expression and Phage Display. PLoS One Biology, in press.
2. Wang, Xiaohu, Haichun Gao, Yufeng Shen, George M. Weinstock, Jizhong Zhou, and Timothy Palzkill. 2008. A High-throughput Percentage-of-Binding Strategy to Measure Binding Energies in DNA-Protein Interactions: Application to Genome Scale Site Discovery. Nucl. Acids Res., 36: 4863-4871
3. Yang, Y.F., Mengxia Zhu, Liyou Wu, and Jizhong Zhou. 2008. Assessment of Data Processing to Improve Reliability of Microarray Experiments Using Genomic DNA Reference. BMC Genomics 2008, 9(Suppl 2):S5doi:10.1186/1471-2164-9-S2-S5.
4. Gao, Haichun, Xiaohu Wang, Zamin K. Yang, Timothy Palzkill, and J.-Z. Zhou. 2008. Probing Regulon of ArcA in Shewanella oneidensis MR-1 by Integrated Genomic Analyses. BMC Genomics, 9: 42 (17 pages)
5. Yang, Yunfeng, Daniel P. Harris, Feng Luo, Liyou Wu, Andrea B. Parsons, Anthony V. Palumbo and J.-Z. Zhou. 2008. Characterization of the Shewanella oneidensis Fur Gene: Roles in Iron and Acid Tolerance Response. BMC Genomics 2008, 9(Suppl 1):S11.
6. Bretschger, O, Anna Obraztsova, Carter A. Sturm, In Seop Chang, Yuri A. Gorby, Samantha B. Reed, David E. Culley, Catherine L. Reardon, Soumitra Barua, Margaret F. Romine, Jizhong Zhou, Alex S. Beliaev, Rachida Bouhenni, Daad Saffarini, Florian Mansfeld, Byung-Hong Kim, James K. Fredrickson, and Kenneth H. Nealson. 2007. An exploration of current production and metal oxide reduction by Shewanella oneidensis MR-1 wild-type and mutants. Appl. Environ. Microbiol., 73:7003-7012.
7. Luo*, F., D. Yang*, J. Zhong*, Haichun Gao, Latifur Khan, Dorothea K. Thompson and Jizhong Zhou. 2007. Constructing gene co-expression networks and predicting functions of unknown genes by random matrix theory. BMC Bioinformatics, 8:299 (17 pages). *Equal contribution.
8. Luo, F., Yunfeng Yang, Chin-Fu Chen, Jizhong Zhou, Richard H. Scheuermann. 2007. Modular Organization of Protein Interaction Networks. Bioinformatics. 23: 207-214.

C. Published papers supported by SF

9. Chourey, K., M. Thompson, J. Morrell-Falvey, N. C. VerBerkmoes, S. D. Brown, M. Shah, J. Zhou, M. Doktycz, R. L. Hettich, and D. K. Thompson. 2006. Global molecular and morphological effects of 24-hour chromium(VI) exposure on Shewanella oneidensis MR-1. Appl. Environ. Microbiol. 72: 6331-6344

10. Gao, Haichun, Anna Obraztova, Nathan Stewart, Radu Popa, James K. Fredrickson, James M. Tiedje, Kenneth, H. Nealson, and Jizhong Zhou. 2006. Shewanella loihica sp. nov., isolated from iron-rich microbial mats in Pacific Ocean. Int J Syst Evol Microbiol ; 56: 1911-1916.
11. Luo, F., J. Zhong, Y. Yang, R. H. Scheuermann, and J.-Z. Zhou. 2006. Application of random matrix theory to biological networks. Physics Letters A: 357: 420-423..
12. Gao, H., Zamin Koo Yang, Liyou, Wu, Dorothea K. Thompson, and J.-Z. Zhou. 2006. Global Transcriptome Analysis of the Cold Shock Response of Shewanella oneidensis MR-1 and mutational analysis of its classical cold-shock proteins. J. Bacteriol., 188:4560-4569.
13. Gao, W., Yongqing Liu, Carol S. Giometti, Liyou Wu, Xueduan Liu, Tinfeng Yan, Dawn Stanek, Dong Xu, Ying Xu, Matthew W. Fields, and J.-Z. Zhou. 2006. Functional annotation of a conserved hypothetical protein in Shewanella oneidensis MR-1: a PHB-like protein involved in iron homeostasis and oxidative damage protection. BMC Genomics, 7:76.
14. Luo, F. Jianxin Zhong, Yunfeng Yang, Jizhong Zhou. 2006. Application of Random Matrix Theory to Microarray Profiles for Discovering Functional Gene Modules. Physics Review E. Physical Review E, 73 (031924), 1-5.
15. Roh, Y, Haichun Gao, Hojatollah Vali, David W. Kennedy, Zamin Yang, Weimin Gao, Alice C. Dohnalkova, Raymond D. Stapleton, Ji-Won Moon, Tommy J. Phelps, James K. Fredrickson, and, J. Zhou. 2006. Metal Reduction and Iron Biomineralization by a Psychrotolerant Fe(III)-Reducing Bacterium Shewanella sp. PV-4. Appl. & Environ. Microbiol. 72:3236-3244.
16. Wan, X.-F., J.-Z. Zhou, and D. Xu. 2006. CodonO: a new informatics method for measuring synonymous codon usage bias within and across genomes. International Journal of General Systems. 35:109-125.
17. Qiu, Xiaoyun, Michael J. Daly, Alexander Vasilenko, Marina V. Omelchenko, E. K. Gaidamakova, Liyou Wu, Jizhong Zhou, George W. Sundin and James M. Tiedje. 2006. Transcriptome Analysis Applied to Survival of Shewanella oneidensis MR-1 Exposed to Ionizing Radiation and Comparison to Deinococcus radiodurans. J. Bacteriol. 188:1199-1204.
18. Windt, W.D, Haichun Gao, W. Kromer, Jan Dick, Nico Boon, J.-Z. Zhou, and Willy Verstraete. 2006. Molecular characterization of an autoaggregating mutant of Shewanella oneidensis. Microbiology, 152: 721-729.
19. Beliaev, Alexander S., Dawn M. Stanek, Joel A. Klappenbach, Liyou Wu, Daad A. Saffarini, Margie F. Romine, Kenneth H. Nealson, Jim K. Fredrickson, and Jizhong Zhou. Electron Acceptor-Induced Shifts in Shewanella oneidensis MR-1 Gene Expression Profiles. J. Bacteriol., 187:7138-7145.
20. Bencheikh-Latmani, R., Sarah Middleton Williams, Lisa Haucke, Craig S. Criddle, Liyou Wu, J.-Z. Zhou, Bradley M. Tebo. 2005. Global Transcriptional Profiling of Shewanella oneidensis MR-1 During Cr(VI) and U(VI) Reduction. Appl. Environ. Microbiol., 71:7453-7460.
21. Qiu, X., G. W. Sundin, L. Wu, J.-Z. Zhou, and J. M. Tiedje. 2005. Comparative analysis of differentially expressed genes in Shewanella oneidensis MR-1 following UVA-, UVB- and UVC exposure. J. Bacteriol, 187: 3556-3564.
22. Stapleton, R., Zakee L. Sabree, Anthony V. Palumbo, Craig Moyer, Allan Devol, Yul Roh, and J.-Z. Zhou. 2005. Metal reduction at cold temperatures by Shewanella isolates from various marine environments. Aquatic Microbiol., 38: 81-91.

23. Zhou, J.-Z., D.K. Thompson, Y. Xu, and James M. Tiedje. 2004. Microbial Functional Genomics. 624pp. John Wiley & Sons, Hoboken, New Jersey. (The first comprehensive book in this field)
24. Kolker, E., Alex F. Picone, Michael Y. Galperin, Margaret F. Romine, Roger Higdon, Kira S. Makarova, Natali Kolker, Gordon A. Anderson, Kenneth J. Auberry, Gyorgy Babnigg, Alex S. Beliaev, Paul Edlefsen, Dwayne A. Elias, Yuri Gorby, Ted Holzman, Joel Klappenbach, Konstantinos T. Konstantinidis, Miriam L. Land, Mary S. Lipton, Lee-Ann McCue, Matthew Monroe, Ljiljana Pasa-Tolic, Grigoriy Pinchuk, Samuel Purvine, Xiaoyun Qiu, Margaret Serres, Sasha Tsapin, Brian A. Zakrajsek, Wenhong Zhu, Jizhong Zhou, Frank W. Larimer, Charles Lawrence, Monica Riley, Frank R. Collart, John R. Yates, III, Richard D. Smith, Carol Giometti, Kenneth Nealson, James K. Fredrickson, and James M. Tiedje. 2005. Global Profiling of Shewanella oneidensis MR-1: Expression of 'Hypothetical' Genes and Improved Functional Annotations. Proc Nat. Acad. Sci. 102: 2099-2104.
25. Wan, X.-F., N.C. VerBerkmoes, L. A. McCue, D. Stanek, H. Connelly, L. Wu, X. Liu, T. Yan, A. Leaphart, R. L. Hettich, J.-Z. Zhou, and D. K. Thompson. 2004. Defining the Shewanella oneidensis FUR Regulon: Integration of Genome-Wide Expression Analysis, Proteome Characterization, and Regulatory Motif Discovery. J. Bacteriol. 186: 8385-8400.
26. Wan, X.-F., D. Xu, Andris Kleinhofs and J.-Z. Zhou. 2004. Quantitative relationship between synonymous codon usage bias and GC composition across the unicellular genomes. BMC Evolutionary Biology 2004, 4:19
27. Luo, F., L. Khan, F. Bastani, I-L. Yen and J.-Z. Zhou. 2004. A Dynamically Growing Self-Organizing Tree (DGSOT) for Hierarchical Clustering Gene Expression Profiles. Bioinformatics, 20: 2605-2617.
28. Wan, X., Xu, D., and J.-Z. Zhou. 2003. A new informatics method for measuring synonymous codon usage bias. In Intelligent engineering systems through artificial neural networks, vol 13, eds. Dagli et al., p1101-1018. New York, NY: ASME Press
29. Yost, C, L. Hauser, F. Larimer, D.K. Thompson, A. Beliaev, J.-Z. Zhou, Y. Xu, and D. Xu. 2003. A computational study of Shewanella oneidensis MR-1: Structural prediction and functional inference of hypothetical proteins. Omics: A Journal of Integrative Biology.7:177-191.
30. Beliaev, A. S, D. K. Thompson, M. Fields, L. Wu, D. P. Lies, K. H. Nealson, and J.-Z. Zhou. 2002.. Microarray Transcription Profiling of a Shewanella oneidensis etrA Mutant. J. Bacteriol. 184: 4612-4616.
31. Xu, D., G. Li, L. Wu, J.-Z. Zhou, and Y. Xu. 2002. PRIMEGENS: A Computer Program for Robust and Efficient Design of Gene-Specific Targets on Microarrays. Bioinformatics, 18:1432-1437, 2002.
32. Zhou, J.-Z., and J. M. Tiedje. 2002. New DOE program expands dramatically beyond microbial genomics. ASM News: 68:110-111.
33. Thompson, D.K, A. S. Beliaev, C. S. Giometti, S. L. Tollaksen, T. Khare, D. P. Lies, Kenneth H. Nealson, H. Lim, J. Yates III, C. C. Brandt, J. M. Tiedje, and J.-Z 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. 68:881-892.
34. Beliaev, A. S., D. K. Thompson, C. S. Giometti, G.-S. Li, J. Yates III, K. H. Nealson, J.M. Tiedje, J. F. Heidelberg, and J.-Z. Zhou. 2002. Gene and Protein Expression Profiles of Shewanella oneidensis During Anaerobic Growth with Different Electron Acceptors. Omics:

A Journal of Integrative Biology 6: 39-60.
35. Murray, A. E., D. Lies, G. Li, K. Nealson, J-Z. Zhou, J. M. Tiedje. 2001. DNA:DNA hybridization to microarrays reveals gene-specific differences between closely related microbial genomes. PNAS, 98: 9853-9858.

D. Papers partially supported by SF but mainly supported by other projects

36. Brown, S.D., M. R. Thompson, N. C. VerBerkmoes, K. Chourey, M. Shah, J.-Z. Zhou, R. L. Hettich, and D. K. Thompson. 2006. Molecular Dynamics of the Shewanella oneidensis Response to Chromate Stress. Molecular and Cellular Proteomics., In press.
37. Leaphart, A. B., D. K. Thompson, K. Huang, Eric Alm, X.-F. Wan A. Arkin, S. D. Brown, L. Wu, T. Yan, X. Liu, G. S. Wickham, and J.-Z. Zhou. 2006. Transcriptome Profiling of Shewanella oneidensis Gene Expression Following Exposure to Acidic and Alkaline pH. J. Bacteriol. 188:1633-42..
38. Brown, S.D, M. Martin, K. Huang, E. Alm, Y. Yang, A. Arkin, J.-Z. Zhou, and D. K. Thompson. Cellular Response of Shewanella oneidensis to Strontium Stress. Appl. Environ. Microbiol., 72: 890900.
39. Liu, Y., W.-M. Gao, T. Yan, L. Wu, X. Liu, E. Alm, S. Wang, A. Arkin, M. W. Fields, D. K. Thompson, and J.-Z. Zhou. 2005. Transcriptome Analysis of Shewanella oneidensis MR-1 in Response to Elevated Salt Conditions. J. Bacteriol, 187: 2901-2907.
40. Gao, W., Y. Liu, J. Zhou, and P. Hongjun. 2005. Effects of a Strong Static Magnetic Field on Bacterium Shewanella oneidensis: An Assessment by Using Whole Genome Microarray. Bioelectromagnetics, 26:2-6.