Final Project Report – December 2008

Project title: The Shewanella Federation: Functional Genomic Investigations of Dissimilatory Metal-Reducing Shewanella

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1. 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.

2. 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. We are analyzing such data now.

3. 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 $\Delta\Delta G$ 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.

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