Project 1010013

# Integrated Analysis of Protein Complexes and Regulatory Networks Involved in Anaerobic Energy Metabolism of Shewanella Oneidensis MR-1

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**RESULTS TO DATE:** I. Progress Summary During the 2005 funding year, our research efforts focused on three primary aspects of S.oneidensis biology important for potential bioremediation applications: 1) nitrate reduction, 2) the stress response, and 3) understanding the diversity within the Shewanella since natural remediating populations comprise more than one phenotype. Research in these areas indicated that S. oneidensis MR-1 has the ability to utilize multiple electron acceptors such as nitrate and oxygen that are often found in environmental redox gradients. Futhermore, S. oneidensis MR-1 is likely required to live in such gradients due to a remarkable sensitivity to oxidative damage induced by nitrite accumulation or UV and ionizing radiation. We also used a new gene deletion mutagenesis method reported last year for deleting genes important to nitrate reduction S. oneidensis MR-1. A list of publications resulting from these efforts is attached and key developments from this research are described below.

II. Objectives and Experimental Approaches A. Project Goals for Michigan State University Research conducted at Michigan State University during 2004 focused on research activities outlined by the following three main goals: 1. Anaerobic Nitrate Reduction 2. Stress Response: Ionizing Radiation and Nitrite Toxicity 3. Using Comparative Genomics to Understand Shewanella Population Diversity

B. Research Progress During Funding Year 2005 The three research objectives described below reflect finishing old goals and beginning work on new goals set forth in our 2004 progress report and renewal.

Objective 1: Anaerobic Nitrate Reduction. Nitrate is an extensive co-contaminant at some DOE sites making metal and radionuclide reduction problematic. Hence, we sought to better understand the nitrate reduction pathway and its control in S. oneidensis MR-1. It is not known whether the nitrate reduction is by denitrification or dissimilatory nitrate reduction into ammonium (DNRA). By both physiological and genetic evidence, we proved that DNRA is the nitrate reduction pathway in this organism. Using the complete genome sequence of S. oneidensis MR-1, we identified a gene encoding a periplasmic nitrate reductase based on its 72% sequence identity with the napA gene in E. coli. Anaerobic growth of MR-1 on nitrate was abolished in a site directed napA mutant, indicating that NapA is the only nitrate reductase present. The anaerobic expression of napA and nrfA, a homolog of the cytochrome b552 nitrite reductase in E. coli, increased with increasing nitrate concentration until a plateau was reached at 3 mM KNO3. This indicates that these genes are not repressed by increasing concentrations of nitrate. The reduction of nitrate can generate intermediates that can be toxic to the microorganism. To determine the genetic response of MR-1 to high concentrations of nitrate, DNA microarrays were used to obtain a complete gene expression profile of MR-1 at low (1 mM) versus high (40 mM) nitrate concentrations. Genes encoding transporters and efflux pumps were up-regulated, perhaps as a mechanism to export toxic compounds. In addition, the gene expression profile of MR-1, grown anaerobically with nitrate as the only electron acceptor, suggested that this dissimilatory pathway contributes to N assimilation. Hence the nitrate reduction pathway could serve a dual purpose. The role of EtrA, a homolog of Fnr (global anaerobic regulator in E. coli) was examined using an etrA deletion mutant we constructed, S. oneidensis EtrA7-1. The global transcriptome suggested a starvation response for anaerobic cultures of Etra7-1 when nitrate was the electron acceptor. Genes invoved in the activation and synthesis of LambdaSo, MuSo1 and MuSo2 prophages of MR-1 were up-regulated, suggesting a phage infection. This could be responsible for the low growth yields observed for EtrA7-1 when compared to the wild type. Starvation is a stress condition that is known to induce viral infections. Even though starvation was not directly targeted for examination, the results in this study suggest that EtrA might play an important in the survival of MR-1 under starvation. Moreover, the low biomass suggests a greater sensitivity of MR-1 to starvation than the

toxicity associated with high nitrate concentrations. Down-regulation of genes involved in the nitrate reduction pathway was also observed for EtrA7-1 relative to the wild type, which suggests a positive regulatory role for this protein in the nitrate reduction pathway of S. oneidensis MR-1.

Objective 2. Stress Response: Ionizing Radiation and Nitrite Toxicity. We previously reported on the basis of the extreme UV and solar radiation sensitive of MR-1 at the transcriptome and physiological level, and have now compared these results to the organisms sensitivity to ionizing radiation. The ionizing radiation (IR) dose that yields 20% survival (D20) of Shewanella oneidensis MR-1 is lower by factors of 20 and 200 than for Escherichia coli and Deinococcus radiodurans, respectively. Whole transcriptome analyses were used to identify the genes of MR-1 responding to 40 Gy (D20). We observed the induction of 170 genes and repression of 87 genes in MR-1 during a one-hour recovery period after irradiation. The genomic response of MR-1 to IR is very similar to its response to ultraviolet radiation (254 nm), which included induction of systems involved in DNA repair and prophage synthesis. In contrast to the radioresistant D. radiodurans, currently the only other bacterium subjected to transcriptome profiling following g-irradiation, differential regulation of tricarboxylic acid cycle activity in MR-1 after IR was not observed and the cells strongly induced antioxidant enzymes during recovery. Since MR-1 exposed to 40 Gy suffers less than one double strand break (DSB) per genome and mounts a strong DNA repair response, DNA damage caused during irradiation might not be the principal cause of death in irradiated cells. Instead, a combination of oxidative stress, protein damage and prophage induced during recovery might underlie this organism?s great sensitivity to radiation. These studies together represent the most comprehensive whole genome comparison of radiation stress yet provided for any organism.

Objective 3. Using Comparative Genomics to Understand Shewanella Population Diversity.

Comparative Genomics. Eleven genomes of Shewanella are being sequenced at the Joint Genome Institute and are at various stages of the gap-closing phase. Most genomes are currently in <3 genomic scaffolds, which greatly facilitates comparative genomic analysis. These genomes represent different species or genotypes within species that thrive in a variety of environments including: freshwater lakes and sediments, different depths and, particularly, at redox interfaces of the water-columns of the Black, Baltic, and North Seas, and subsurface sandstone in New Mexico. Therefore, the comparative analysis of these genomic sequences is expected to shed novel insights into the genetic differences that account for ecological success in different environments and provide the basis for different physiologies.

Our preliminary results reveal (as anticipated in the original sequencing proposal by our group) that these 11 genomes form a continuing gradient of evolutionary relatedness, since they include genomes from the same species, closely related species, as well as more distantly related species. This gradient is unprecedented (e.g., the pathogenic Escherichia-Salmonella, the best-sampled group with genomic sequences, has only strains of the same species sequenced) and thus, future analyses of the Shewanella genomes are expected to advance cornerstone issues for microbiology such as the species concept, the species core hypothesis (Lan & Reeves, 2001), gene/genome evolution and functional diversification. Our preliminary findings towards these directions suggest that the current species definition for bacteria does not correspond to distinct genetic clusters in the Shewanella case, rather it corresponds to a continuing gradient of genetic relatedness. Furthermore, among the genomes of the same species such as the S. putrefaciens strain CN32 and W3-18-1, we find major genetic differences (~0.5Mb of sequence is different) and the majority of these differences appear to be ecologically important, suggesting that these genomes represent distinct ecotypes if not species. Consistent with this extensive gene diversity, we find that the conserved gene core for all 11 Shewanella genomes to be ~2,000 genes, which less than half of the genes a typical Shewanella possesses. Furthermore, this core gene set is heavily biased towards informational, cellular processes and central metabolism only (e.g., secondary metabolism, energy production, membrane genes are poorly represented in the core gene set). On the other hand, the Shewanella strain-specific genes, unlike in the E. coli case where these strain differences are largely prophage associated genes and mobile elements, are enriched in functional genes (metabolic pathways and energy-transport related proteins). In other words, it appears that each lineage from the ones sampled with genomic sequences has taken its own route of evolution and has presumably employed a different gene complement for its environmental survival and to perform the important in-situ physiologies.

These results also suggest that bacterial families should more solidly be defined based on evolutionary as opposed to phenotypic characteristics. Ongoing efforts target the study of genome-specific genes that support the ecological distinctiveness of each genome as well as the relationship between gene presence and expression for sequenced Shewanella.

Comparative Proteomics. With the inability to easily distinguish morphological features of bacteria as with larger macro-organisms, rRNA gene surveys have been used to assess organismal diversity of bacteria. From the very first molecular approaches to quantify microbial diversity and community structure over 15 years ago, researchers have consistently recovered large clusters of closely related rRNA gene sequences from a majority of environments. Recent attempts to explain this long-observed phenomenon indicate that this rRNA sequence ?micro-diversity? is a real feature of bacterial populations, and not an artifact of PCR or a consequence of intragenomic rRNA gene redundancy. High-levels of genome sequence heterogeneity in metagenomics provide confirmation that bacterial species exist in nature as populations of diverse genomic compositions. It is unknown, however, whether these substantial intra-species genomic differences are translated by the cell and therefore part of the cell proteome. Hence, we have collaborated with PNNL on comparative proteomic analysis of the twelve strains of S. baltica to determine the core proteome in a natural population of the same Shewanella species to identify the evolutionary and ecological forces creating structuring of the proteome within this population.

These strains were isolated from three different ecological zones in the water column: the oxic zone, oxic-anoxic transition zone, and the anoxic zone. The S. baltica strains are identical in rRNA gene sequence, and they differ significantly in genome content and organization (differ by up to 30% at the DNA-DNA reassociation level). One strain of S. baltica, strain OS155, has been sequenced to 8X coverage (~200 contigs >2 kb) at JGI under the DOE Microbial Genomes program and has a genome size of 5.1 Mbp with 4734 ORFs, and a %G+C content of 46.2%. A complete genome sequence is available for S. oneidensis MR-1 that contains orthologs for 72.5% of the S. baltica OS155 genes, and these strains share 85% average nucleotide identity. All strains were grown under identical conditions and harvested in mid-log phase. Proteomic analysis has just been completed by PNNL and data analyses will be underway shortly. To our knowledge, this is the first application of whole proteome mass spectroscopy data to comparative microbial taxonomy.

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