Shewanella Federation

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

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Shewanella oneidensis MR-1 is a motile, facultative γ-*Proteobacterium* with remarkable metabolic versatility in regards to electron acceptor utilization; it can utilize O_2, nitrate, fumarate, TMAO, DMSO, Mn, Fe, and S^0 as terminal electron acceptors during anaerobic respiration. The ability to effectively reduce nitrate, polyvalent metals including solid phase Fe and Mn oxides and radionuclides such as uranium and technetium has generated considerable interest in the potential role of this organism in metal biogeochemical cycling and bioremediation. The *Shewanella* Federation (SF), a collaborative scientific team assembled by DOE, is applying these approaches to achieve a system-level understanding of how *Shewanella* regulates energy and material flow and to utilize its versatile electron transport system to reduce metals and nitrate. The SF has developed an integrated approach to *Shewanella* functional genomics that capitalizes on the relative strengths, capabilities, and expertise of each group. SF members share information and resources and collaborate on projects that range from a few investigators focused on a defined topic to more complex "Federation-level" efforts. These intend to utilize combined SF capabilities in addressing more general scientific questions.

The SF is organized into various working groups that are engaged in a series of *Shewanella*-based collaborative sub-projects. The ongoing investigations include: **(i)** generation of deletion mutants in each of the 40 predicted *c*-type cytochromes in the MR-1 genome and characterization of their phenotype; **(ii)** global expression profiling using whole genome microarray and proteomic analyses and network modeling of steady-state and transitions between growth on various electron acceptors; and **(iii)** analyses of global regulatory mutants involved in carbon and energy metabolism; and development of common standards and statistical models for numerous SF-wide studies. In addition, coordinate work is underway to revise the annotation of MR-1 based on new information generated by the SF and available in public databases. Supported by the database development, this re-annotation effort will provide the foundation for further functional experimentation, analysis, and modeling of MR-1. Additionally, it will facilitate comparative genomic of analyses of seven new strains of *Shewanella* recently completed by DOE's Joint Genome Institute. These include *S. amazonensis*, *S. putrefaciens* strains CN32 and 200, *S. denitrificans* OS217T, *S. baltica* OS155, *S. frigidimarina* 400, *S. sp.* str. PV-4. In parallel, detailed physiological analyses are also being conducted with the sequenced strains to provide a basis for genomic comparisons and predictions. The overall goal of this collaborative effort is to develop an evolutionary model for speciation in *Shewanella* as well as providing additional insights into electron transport and carbon metabolism.

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Global Profiling of *Shewanella oneidensis* MR-1: Expression of 'Hypothetical' Genes and Improved Functional Annotations

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The γ-proteobacterium *Shewanella oneidensis* strain MR-1 is a metabolically versatile organism that can reduce a wide range of organic compounds, metal ions, and radionuclides. Similar to most other sequenced organisms, approximately 40% of the predicted ORFs in the *S. oneidensis* genome were annotated as uncharacterized 'hypothetical' genes. We implemented an integrative approach using experimental and computational analyses to provide more detailed insight into gene function. Global expression profiles were determined for cells following UV irradiation and under aerobic and suboxic growth conditions. Transcriptomic and proteomic analyses confidently identified 538 'hypothetical' genes as expressed in *S. oneidensis* cells both as mRNAs and proteins (33% of all predicted 'hypothetical' proteins). Publicly available analysis tools and databases and the expression data were applied to improve the annotation of these genes. The annotation results were scored using a seven-category schema that ranked both confidence and precision of the functional assignment. We were able to identify homologs for nearly all of these 'hypothetical' proteins (97%), but could confidently assign exact biochemical functions for only 16 proteins (category 1; 3%). Altogether, computational and experimental evidence provided functional assignments or insights for 240 more genes (categories 2-5; 45%). These functional annotations advance our understanding of genes involved in vital cellular processes including energy conversion, ion transport, secondary metabolism, and signal transduction. We propose that this integrative approach offers a valuable means to undertake the enormous challenge of characterizing the rapidly growing number of 'hypothetical' proteins with each newly sequenced genome.

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Respiratory Pathways and Regulatory Networks of *Shewanella oneidensis* Involved in Energy Metabolism and Environmental Sensing

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Shewanella oneidensis MR-1 is a facultative γ-Proteobacterium with remarkable metabolic versatility in regards to electron acceptor utilization; it can utilize O_2, nitrate, fumarate, Mn, Fe, and S^0 as terminal electron acceptors during respiration. This versatility allows MR-1 to efficiently compete for resources in environments where electron acceptor type and concentration fluctuate in space and time. The ability to effectively reduce polyvalent metals and radionuclides, including solid phase Fe and Mn oxides, has generated considerable interest in the potential role of this organism in biogeochemical cycling and in the bioremediation of contaminant metals and radionuclides. The entire genome sequence of MR-1 has been determined and high throughput methods for measuring gene expression are being developed and applied. This project is part of the *Shewanella* Federation, a multi-investigator and cross-institutional consortium formed to achieve a systems level understanding of how *S. oneidensis* MR-1 senses and responds to its environment.

Electron Acceptor-Induced Shifts in *S. oneidensis* MR-1 Gene Expression Profiles. To define the repertoire of genes responding to both metal and non-metal electron acceptors and identify basic regulatory mechanisms governing anaerobic respiration in *S. oneidensis*, we compared mRNA expression patterns of anaerobic cultures incubated with fumarate to those exposed to nitrate, thiosulfate, DMSO, TMAO, ferric citrate, hydrous HFO, manganese dioxide, colloidal manganese, and cobalt using whole-genome arrays. The extent of *S. oneidensis* transcriptome response to metal electron acceptors was revealed by hierarchical clustering analysis, where a high degree of similarity in global expression profiles was exhibited throughout all metal-reducing conditions which resulted in metals grouping separately from non-metal electron acceptors and forming a tight, well-defined branch. In accordance with the results of a principal component analysis, we identified two major expression groups that displayed activation and repression in the presence of metals and accounted for over 60% of the differentially expressed genes. While genes encoding hypothetical and conserved hypothetical proteins dominated both clusters, there were several functional subgroups encoding putative components of electron transport chain, transcriptional regulators and detoxification/toxin resistance proteins that were characterized by their non-specific upregulation to all metal electron acceptors. Contrary to what was expected, the *mtrCAB* operon which encodes two deca-heme *c*-type cytochromes and an outer membrane protein essential for Fe(III) and Mn(IV) respiration in *S. oneidensis* showed 2- to 8- fold decrease in mRNA levels under metal-reducing conditions. In contrast, *S. oneidensis* demonstrated specific transcriptome responses to individual non-metal electron acceptors producing unique clusters of nitrate, thiosulfate and TMAO induced genes. While these observations undoubtedly reflect the nature of metal and non-metal electron acceptors, the diversification and tighter regulatory control of the non-metal respiratory systems may be indicative of different evolutionary pathways taken by these respiratory systems. Moreover, the absence of upregulation for known genes involved in metal reduction may be due to the low-specificity and the opportunistic nature of the metal reduction pathways in *S. oneidensis*. This work represents an important step to-

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wards understanding the anaerobic respiratory system of *S. oneidensis* MR-1 on a genomic scale and has yielded numerous candidate genes for more detailed functional analysis.

Autoaggregation of *Shewanella oneidensis* in Response to High Oxygen Concentrations. Despite the potential environmental importance of this phenomenon, little is known about the mechanisms inducing aggregate formation and subsequent impacts on cells inside the aggregates. Under aerobic conditions, *S. oneidensis* cells are highly adhesive to glass and in the presence of $CaCl_2$ the cells aggregate into large multi-cellular structures. Microscopic analyses of these aggregates identified a presence of DNA, proteins and carbohydrate-like material in the extracellular matrix. In contrast, cells grown under suboxic conditions did not display any autoaggregation while their adhesion to surfaces was significantly reduced. Microarray expression analysis comparing samples of suboxically- vs. aerobically-grown cells identified a set of genes encoding cell-to-cell and cell-to-surface adhesion and colonization factors that positively responded to increased O_2 concentrations. Of particular interest was the O_2-dependent upregulation of *S. oneidensis csgAB* and *csgDEF* operons which are putatively involved in curli fimbrae formation. In other organisms, such *Escherichia coli*, these structures confer attachment to inert surfaces such as glass and have also been implicated in cell-cell attachment. Although, when compared to suboxic conditions, both flocculated and unflocculated cells displayed some similarities in gene expression in response to elevated levels of O_2, autoaggregation had a significant impact on gene expression in *S. oneidensis*. Direct comparison of aggregated versus unagreggated cells grown under 50% dissolved O_2 tension (DOT) revealed remarkable differences in mRNA patterns between these two states. Unflocculated cells displayed significant increase of mRNA levels of genes involved in aerobic energy metabolism, intermediary carbon metabolism and gluconeogenesis as well as chemotaxis and motility. In contrast, several genes putatively involved in anaerobic metabolism, gene cluster encoding outer membrane proteins and cytochromes, and transcriptional regulation were upregulated under 50% DOT aggregated conditions. Remarkably, the majority (~90%) of genes located on the 50-kb megaplasmid of MR-1 displayed substantial levels of upregulation in flocculated cells. It is currently unclear whether this phenomenon is due to a global regulatory effect or to an increase in plasmid copy number. Although further studies are required for resolution, we speculate that autoaggregation in *S. oneidensis* MR-1 may serve as a mechanism to facilitate reduced O_2 tensions within aggregate, leading to the expression of anaerobic genes under bulk aerobic conditions.

Cyclic AMP Signaling and cAMP Receptor Protein-Dependent Regulation of Anaerobic Energy Metabolism in *Shewanella oneidensis* MR-1. Unlike many bacteria studied to date, the ability of *S. oneidensis* to grow anaerobically with several electron acceptors is regulated by the cAMP-receptor protein (CRP). CRP-deficient mutants of MR-1 are impaired in anaerobic reduction and growth with Fe(III), Mn(IV), fumarate, nitrate, and DMSO. Loss of anaerobic respiration in *crp* mutants is due to loss of terminal anaerobic reductases and not due to deficiency in carbon metabolism. To further elucidate the role of CRP and to understand the mechanisms of cAMP-dependent gene expression under anaerobic conditions in *S. oneidensis*, DNA microarray analyses were performed. Comparison of mRNA expression profiles of wild-type and *crp* mutant cells grown anaerobically with different electron acceptors indicated that CRP positively regulates the expression of genes involved in energy generation and transcriptional regulation. These include the periplasmic nitrate reductase, the polysulfide reductase, anaerobic DMSO reductase genes, as well as the nitrate/nitrite sensor protein *narQ*. To identify the mechanisms and proteins that lead to CRP activation under anaerobic conditions, genes predicted to encode adenylate cyclases were analyzed. The genome sequence of *S. oneidensis* contains three putative adenylate cyclase genes, designated *cyaA*, *cyaB*, and *cyaC*. Deletion of *cyaA* or *cyaB* did not affect anaerobic growth with any of the electron acceptors

tested while deletion of *cyaC* resulted in growth deficiency with DMSO. Surprisingly, deletions of both *cyaA* and *cyaC* resulted in anaerobic growth deficiency with DMSO, nitrate, Fe(III), Mn(IV), and fumarate. These phenotypes are similar to the phenotypes of the CRP-deficient mutants. Our results indicate that both CyaA and CyaC are needed for the production of cAMP under anaerobic conditions, and for activation of CRP. Further work to identify the cAMP signaling pathways in *S. oneidensis* is underway.

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Functional Analysis of *Shewanella*, A Cross Genome Comparison

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The genome sequence of *Shewanella oneidensis* MR-1, a microbe with unique metabolic and respiratory properties including the use of metals as electron acceptors, has been published (Heidelberg et al. 2002). Recently six additional *Shewanella* genomes; *S. putrefaciens* CN-32, *S. alga* PV-4, *S. amazonensis* SB2B, *S. baltica* OS155, *S. frigidimarina* NCIMB400, and *S. denitrificans* OS2717 have been sequenced at the Joint Genome Institute. The new strains were selected as representatives of different ecological niches and redox environments ranging from terrestrial to marine and freshwater sediments. We are making use of the new sequences to do a comparative analysis between the protein coding sequences of *S. oneidensis* and MR-1 and those of the newly available *Shewanella* strains. In addition we are including the genomes of *Escherichia coli*, an experimentally well studied organism, and *Geobacter sulfurreducens*, an organism with metal reducing capabilities. Our aim is to detect variations in the protein content that may be related to differences in metabolic properties and environmental adaptation for the organisms.

In our previous work we have assembled groups of sequence similar proteins of *S. oneidensis* MR-1. We initially identified fused proteins (155) as these complicate the grouping process and separated them into stand-alone functional entities. The Darwin algorithm was used to detect sequence similarity among the protein sequences. A transitive grouping process was applied to generate sequence similar groups containing both closely related as well as more distantly related members. We also restrict the membership of a protein to one sequence similar group. As a result we identified 406 paralogous or sequence similar groups with memberships ranging from 2 to 64. The largest paralogous groups were found to encode for response regulators, ATP-binding component of the ABC superfamily transporters, transcriptional regulators of the LysR family, regulatory proteins, and sensory histidine kinases. The group sizes show a power-law distribution with most of the groups having few members and few groups having many members. The paralogous groups represent ancestral genes which have gone through duplication and divergence to generate today's gene families. These gene families encode for proteins with related functions. Gene duplication and divergence is thought of as an important means by which an organism may specialize or generate functions.

We are making use of the paralogous group data for the *S. oneidensis* MR-1 genome to search for sequence matches in the newly sequenced *Shewanella* genomes and in *E. coli* and *G. sulfurreducens*. Data will be presented on the distribution of homologs to the members of the *S. oneidensis* MR-1 paralogous groups. The data will be analyzed to identify differences or similarities between the or-

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ganisms and further use this information to shed light on functions that may be of importance to the metabolic properties and environmental fitness of the organisms.

One of the larger *S. oneidensis* MR-1 paralogous groups was found to contain a family of 27 methyl-accepting chemotaxis proteins (MCPs). These proteins are located in the membrane where they serve as signal receptors for the chemotaxis apparatus. Five MCPs are encoded in the *E. coli* genome and they are known to bind specific attractants or repellants. The expanded repertoire of MCPs in *S. oneidensis* MR-1 suggests that the chemotactic response and environmental sensing is highly specialized in this organism. Sequence similarity between the *S. oneidensis* MR-1 MCPs and the proteins encoded in the newly sequenced *Shewanella* strains shows an expansion of the MCPs versus that of *E. coli* for all the strains. Interestingly the number of proteins with sequence matches varies widely in the different genomes from 19 to 40. *G. sulfurreducens* appears to contain 33 homologs of the *S. oneidensis* MR-1 MCP group. The sequence similarity and distribution of other chemotaxis related proteins will be presented.

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Optical Methods for Characterization of Expression Levels and Protein-Protein Interactions in *Shewanella oneidensis* MR-1

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Biological networks are dependent on a delicate balance of cellular signaling and dynamic transcriptional response, and it has become increasingly important to unravel these networks by accurately quantifying gene expression levels and mapping protein-protein interactions. We have developed a single optical technique, Alternating Laser Excitation (ALEX), which can integrate the analysis of protein-protein interactions and gene expression levels in a sensitive and potentially high-throughput manner.

A protein-protein interaction rich system in MR-1 is transcriptional regulation. Using the sigma factor, σ^{24}, and the DNA bending protein IHF, we are currently reconstructing an active transcription system from MR-1 and creating fluorescently labeled proteins for interaction analysis. When fluorescently labeled proteins are characterized, the ALEX method can be used to examine the mechanistic process of gene regulation by σ^{24}-RNA polymerase (RNAP) from the formation of open complex to transcription elongation, and the dynamics of DNA bending by IHF for transcriptional initiation can be resolved.

Additionally, we are currently expanding the capabilities of the ALEX technique for gene expression analysis. To demonstrate advances in this effort, we examined a DNA model system using two-color coincident detection. By hybridizing two spectrally distinct fluorophores to the target, we detected and quantified individual DNA molecules. Hybridized complexes were detected by colocalization of the probes to the target and specificity of the probes was determined by Forster resonance energy transfer (FRET) between the probes. With ALEX, we have detected and quantified interactions at the DNA level and are currently focusing our efforts at the mRNA level. Progress in both quantification of gene expression and protein-protein interactions will be reported.

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Reverse-Engineering Microbial Networks in *Escherichia coli* and *Shewanella oneidensis* MR-1 via Large-Scale Perturbation Studies

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The impressive capabilities of microbes, ranging from energy transduction to signal processing, rival those of any engineered system. Functions of respiration, growth, and environmental sensing are principally regulated by transcriptional gene networks. Identifying the large-scale structure and dynamics of such networks is an important first step towards engineering microbes for applications in bioremediation and energy production.

Towards this goal, we have recently completed a pilot study validating an approach for rapid cell-wide reverse-engineering of transcriptional gene networks. In an extended study of the DNA-damage response network of *Escherichia coli*, we generated genome expression profiles of cells under 65 experimental conditions, encompassing both time-series profiles and genetic perturbations, in a background of antibiotic-induced DNA damage.

We succeeded in reconstructing a network map comprising over one hundred genes using an inference algorithm developed previously in our lab [1,2]. This network provides a comprehensive picture of a major stress-response system in prokaryotes, buttressing and unifying evidence from previous studies. In addition, we have also identified several novel regulators in the network, for which we are pursuing further experimental validation. Our results establish the feasibility and scalability of our reverse-engineering approach, and have laid the groundwork for a similar study in *Shewanella oneidensis* MR-1.

S. oneidensis is a gram-negative microbe whose ability to reduce heavy-metals and other organic toxins has made it a promising candidate for use in environmental remediation. Using our reverse-engineering methods, we are conducting a broad series of growth-condition perturbations to reconstruct the transcriptional networks governing *Shewanella*'s respiratory system. To facilitate these studies, we have designed the first high-density Affymetrix oligonucleotide microarray for *S. oneidensis*. Beyond its use for genome-wide expression profiling, this microarray can also be used in chromatin immunoprecipitation studies, which will complement our expression-based inference techniques.

The knowledge we are deriving from our work both in *E. coli* and *S. oneidensis* has a variety of applications, including the improvement of antibiotics, environmental remediation, and the prospect of biologically-derived energy sources.

References

1. Gardner TS, di Bernardo D, Lorenz D, Collins JJ. Inferring genetic networks and identifying compound mode of action via expression profiling. Science. 2003 Jul 4;301(5629):102-5.
2. Tegner J, Yeung MK, Hasty J, Collins JJ. Reverse engineering gene networks: Integrating genetic perturbations with dynamical modeling. Proc Natl Acad Sci U S A. 2003 May 13;100(10):5944-9.

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Comparative Analysis of Gene Expression Profiles of *Shewanella oneidensis* MR-1 Following Exposure to Ionizing Radiation and Ultraviolet Radiation

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Shewanella oneidensis MR-1, a Gamma proteobacterium, is notable in the terminal electron acceptors it uses including some toxic metal ions and radionuclides. Thus it has potential for bioremediation. However, MR-1 is highly sensitive to both ionizing radiation (IR) and ultraviolet radiation (UVR). We delineated the genomic response of *Shewanella oneidensis* MR-1 to gamma ray, UVC (254 nm), UVB (290-320 nm), UVA (320-400 nm) and natural solar radiation. A total of 5.9-, 4.2-, 3.9-, 8.1-, and 28.0% of the MR-1 genome showed differential expression ($P<0.05$ and fold >2), respectively, at a dose that yielded about 20% survival rate. The gene expression profile of MR-1 in response to ionizing radiation is more similar to that of UVC, which is characterized by the induction of SOS response and prophage synthesis, plus a strong induction of antioxidant enzymes. Genomic response to UVB is a combination of the UVC and UVA patterns, which represents a shift from shorter wavelength of UVR-induced direct DNA damage and activation of prophages to longer wavelength of UVR-induced global photo-oxidative damage. We observed the traditional UVA-induced stress responses in MR-1 such as induction of antioxidant enzymes and proteins, sequestration of the transition metals and activation of the degradative pathways, however, the induction of heavy metal and multidrug efflux pumps is a previously unknown phenotype for this stress. Consistent with natural solar UV radiation composition (about 95% UVA and 5% UVB), genomic response to solar radiation is more similar to that of UVA but with more genes induced for detoxification. In addition, the number of differentially expressed genes from most functional categories was much greater than for UVB or UVA or their sum. This unique gene expression profile indicates that natural solar radiation impacts biological processes in a much more complex way than previously thought.

Comparative genome analysis indicates that *S. oneidensis* MR-1 encodes a complex set of DNA repair and detoxification genes. For example, about 2.8% of the MR-1 genome is dedicated to DNA repair, replication and recombination, which is very similar to that of *Escherichia coli* K12 (2.7%) and the extremely radiation resistant *Deinococcus radiodurans* R1 (3.1%). However, only about 5.8- and 13.9% of those genes were induced in MR-1 by UVC and gamma ray, respectively, which is much lower than in *E. coli* K12 (15.7% were induced by UVC) and in *D. radiodurans* (22.0% were induced by gamma ray). This result indicates that alteration in gene content and gene regulation, which may be the consequence of lack of recent natural selection, contribute to the high radiation sensitivity of MR-1.

Although we observed a strong induction of the SOS response in MR-1 following exposure to IR or short wavelength UVR (UVC and UVB), DNA damage caused during irradiation itself might not be the primary cause of cell death since there is relatively little DNA damage in MR-1 following 40 Gy or 3.3 J m^{-2} of UVC. MR-1 is a respiratory generalist and is very rich in cytochromes, which together with other respiratory chain components such as flavins and quinones are an important

source of oxidative stress. A recent study by Daly et al. showed that in contrast to *D. radiodurans*, MR-1 accumulates exceptionally high Fe and low Mn levels. Accumulation of Mn in bacteria has been proposed to serve as non-enzymatic antioxidants during recovery after radiation exposure. Collectively, the results support that irradiated *S. oneidensis* is responding to oxidative stress elicited by metabolism-induced free radicals produced during recovery. For Fe-rich, Mn-poor cells such as *S. oneidensis*, death at low doses of IR might be caused by a combination of oxidative stress and the induction of lytic prophages, as proposed following recovery from UV radiation.

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The Microbial Proteome Project: A Database of Microbial Protein Expression in the Context of Genome Analysis

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This project, funded through the Microbial Genome and Genomics:GTL programs, is focused on the detection and characterization of differential protein expression in microbial systems relevant to the goals of the Office of Biological and Environmental Research. As part of this effort, relational databases are being used to assimilate and integrate the data collected. This growing knowledgebase can serve as a resource for comparison of proteomes across species and assessment of differential cellular responses to a variety of growth conditions. The Microbial Proteome Project knowledgebase currently includes experimental details and proteome data for 11 different microbes (*Deinococcus radiodurans, Geobacter sulfurreducens, Geobacter metallireducens, Methanococcus jannaschii, Prochlorococcus marinus, Pyrococcus furiosus, Psychrobacter sp.5*, *Rhodopseudomonas palustris, Rhodobacter sphaeroides, Shewanella oneidensis,* and *Synechocystis sp. PC*) and includes over 8000 protein pattern images that are accessible to authenticated users.

As partners in the *Shewanella* Federation and University of Massachusetts Genomics:GTL projects, our laboratory efforts in the past year have been focused on the proteomes of *S. oneidensis MR-1*, *Geobacter sulfurreducens*, and *Geobacter metallireducens*. Using two-dimensional electrophoresis (2DE) analyses, we have been able to complement the results generated using LC/MS-MS proteomics and microarray mRNA analysis methods, providing "snapshots" of protein components to confirm the expression of specific proteins, measure their relative abundance, and detect post-translational modifications. In addition to traditional 2DE methods, we have introduced approaches that capitalize on the sensitivity of fluorescence and chemiluminescence. Using these approaches, we have been able to detect differences in protein phosphorylation patterns and in heme protein expression. By combining affinity chromatography to enrich samples for phosphoproteins with detection using phosphate-specific fluorescent dyes and immunoblotting with antibodies against specific phosphoamino acids, we have detected differences in the phosphoproteome of *S. oneidensis* when cells are grown aerobically compared to anaerobically. To optimize our detection of *c*-type cytochromes, we have used an isoelectric focusing fractionation protocol to concentrate *G. sulfurreducens* and *G. metallireducens* protein samples prior to electrophoresis and then detected the heme-positive proteins using a chemiluminescence assay. This latter method has increased the number of heme-positive proteins detectable from

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only four or five up to approximately 25 for each *Geobacter* species, and has provided the opportunity to do comparative analysis of the *c*-type cytochromes expressed by each of these microbes grown under different conditions.

In parallel with our investigation of subsets of the microbial proteomes, we continued our efforts to identify both constitutively expressed and induced proteins in *S. oneidensis*, *G. sulfurreducens*, and *G. metallireducens*. Our knowledgebase currently includes the identifications of 575 *S. oneidensis* MR-1, 400 *G. sulfurreducens* PCA, and 77 *G. metallireducens* proteins together with the peptide mass data used to obtain these identifications. These identifications represent the most abundant protein spots detected in 2DE patterns with either Coomassie Blue R250 or silver nitrate and include proteins expressed in *S. oneidensis* cells grown with 50% dissolved oxygen (i.e., aerobic conditions), 0% dissolved oxygen (i.e., suboxic or hypoxic), Fe(III), nitrate, or fumarate, *G. sulfurreducens* cells grown with either fumarate or Fe(III), and *G. metallireducens* cells grown with either nitrate or Fe(III). When the functional categories of the identified *S. oneidensis* and *G. sulfurreducens* proteins are compared, the relative number of identified proteins in each category is quite similar. Exceptions are found in the categories of cellular processes and protein fate with a greater percentage of *S. oneidensis* proteins identified than *G. sulfurreducens* proteins. More proteins associated with regulatory functions, on the other hand, have been identified among the *G. sulfurreducens* proteins detected in 2DE patterns than among the *S. oneidensis* proteins. Also, a larger number of *G. sulfurreducens* proteins with no annotation or annotated as having unknown function have been identified whereas more proteins annotated as hypothetical proteins has been identified in the 2DE patterns of *S. oneidensis*. The latter observation could reflect the less mature annotation of the *G. sulfurreducens* genome compared to the *S. oneidensis* genome, i.e., proteins currently having no functional annotation could be reannotated as proteins with known function as improved methods for functional annotations become available. Of the 77 *G. metallireducens* proteins identified, 25 are currently annotated as unknown, hypothetical, or conserved hypothetical proteins.

Since the overall goal of this project is to provide a public resource of protein expression information for microbes in the context of genome sequence, in addition to a secure website shared by *Shewanella* and *Geobacter* co-investigators, public websites have been designed to provide access to proteome analysis results as they become validated and published. ProteomeWeb (http://ProteomeWeb.anl.gov) is an interactive public site that currently provides the identification of *M. jannaschii* proteins detected by 2DE with links to genome sequence information, tools for mining the proteome data, and links to metabolic pathways. GelBank (http://GelBank.anl.gov) includes the complete genome sequences of approximately 205 microbes and is designed to allow queries of proteome information. Numerous tools are provided, including the capability to search available sequence databases for specific protein functions and amino acid sequences. Web applications pertinent to 2DE analysis are provided on this site (e.g., titration curves for collections of proteins, 2DE pattern animations). The database is currently populated with protein identifications from the Argonne Microbial Proteomics studies and will accept data input from outside users interested in sharing and comparing results from proteome experiments.

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