Title: Construction of Whole Genome Microarrays, and Expression Analysis of *Desulfovibrio vulgaris* cells in Metal-Reducing Conditions
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Final Report

Results: We continue to utilize the oligonucleotide microarrays that were constructed through funding with this project to characterize growth responses of *Desulfovibrio vulgaris* relevant to metal-reducing conditions. To effectively immobilize heavy metals and radionuclides via sulfate-reduction, it is important to understand the cellular responses to adverse factors observed at contaminated subsurface environments (e.g., nutrients, pH, contaminants, growth requirements and products).

One of the major goals of the project is to construct whole-genome microarrays for *Desulfovibrio vulgaris*. First, in order to experimentally establish the criteria for designing gene-specific oligonucleotide probes, an oligonucleotide array was constructed that contained perfect match (PM) and mismatch (MM) probes (50mers and 70mers) based upon 4 genes. The effects of probe-target identity, continuous stretch, mismatch position, and hybridization free energy on specificity were examined. Little hybridization was observed at a probe-target identity of <85% for both 50mer and 70mer probes. 33 to 48% of the PM signal intensities were detected at a probe-target identity of 94% for 50mer oligonucleotides, and 43 to 55% for 70mer probes at a probe-target identity of 96%. When the effects of sequence identity and continuous stretch were considered independently, a stretch probe (>15 bases) contributed an additional 9% of the PM signal intensity compared to a non-stretch probe (< 15 bases) at the same identity level. Cross-hybridization increased as the length of continuous stretch increased. A 35-base stretch for 50mer probes or a 50-base stretch for 70mer probes had approximately 55% of the PM signal. Mismatches should be as close to the middle position of an oligonucleotide probe as possible to minimize cross-hybridization. Little cross-hybridization was observed for probes with a minimal binding free energy greater than -30 kcal/mol for 50mer probes or -40 kcal/mol for 70mer probes. Based on the experimental results, a set of criteria were suggested for the design of gene-specific and group-specific oligonucleotide probes, and these criteria should provide valuable information for the development of new software and algorithms for microarray-based studies.

Secondly, in order to empirically determine the effect of probe length on signal intensities, microarrays with oligonucleotides of different lengths were used to monitor gene expression at a whole genome level. To determine what length of oligonucleotide is a better alternative to PCR-generated probes, the performance of oligonucleotide probes was systematically compared to that of their PCR-generated counterparts for 96 genes from *Shewanella oneidensis* MR-1 in terms of overall signal intensity, numbers of detected genes, specificity, sensitivity and differential gene expression under experimental conditions. Hybridizations conducted at 42^{o}C, 45^{o}C, 50^{o}C, and 60^{o}C indicated that good sensitivities were obtained at 45^{o}C for oligonucleotide probes in the presence of 50% formamide, under which conditions specific signals were detected by both PCR and oligonucleotide probes. Signal intensities increased as the length of

oligonucleotide probes increased, and the 70mer oligonucleotide probes produced similar signal intensities and detected a similar number of ORFs compared to the PCR probes. cDNA, 70mer, 60mer and 50mer arrays had detection sensitivities at 5.0, 25, 100 and 100 ng of genomic DNA, or an approximately equivalent of 1.9×10^6, 9.2×10^6, 3.7×10^7 and 3.7×10^7 copies, respectively when the array was hybridized with genomic DNA. To evaluate differential gene expression under experimental conditions, *S. oneidensis* MR-1 cells were exposed to low or high pH conditions for 30 and 60 min, and the transcriptional profiling detected by oligonucleotide probes (50mer, 60mer, and 70mer) was closely correlated with that detected by the PCR probes. The results demonstrated that 70mer oligonucleotides can achieve the most comparable performance with PCR-generated probes.

We have analyzed expression data as *D. vulgaris* transitioned during electron donor depletion. As the cells transitioned from exponential to stationary-phase a majority of the down-expressed genes were involved in translation and transcription, and this trend continued in the remaining time points. Interestingly, most phage-related genes were up-expressed at the onset of stationary-phase. This result suggested that nutrient depletion may impact community dynamics and DNA transfer mechanisms of sulfate-reducing bacteria via phage cycle. The putative *feo*AB system (in addition to other presumptive iron metabolism genes) was significantly up-expressed, and suggested the possible importance of Fe^{2+} acquisition under metal-reducing conditions. Namely, that iron availability should be considered when sulfate-reducing conditions are stimulated in the subsurface for heavy metal reduction. A large subset of carbohydrate-related genes had altered gene expression, and the total cellular carbohydrate levels declined during the growth phase transition. The carbohydrate data is related to our current project that studies biofilm formation in *D. vulgaris*. Interestingly, the *D. vulgaris* genome does not contain a putative *rpo*S gene, a common attribute of the δ-*Proteobacteria* genomes sequenced to date, and other putative *rpo* genes did not have significantly altered transcription profiles. These data suggested that rpoS is not a universal transcriptional factor for bacteria, and alternative mechanisms exist for the regulation of stasis response in different microorganisms. The results indicated that a subset of approximately 110 genes were uniquely up-expressed as the cells transitioned to stationary-phase (14 on the megaplasmid) and these results suggested an important(s) roles for predicted proteins encoded by the megaplasmid for growth as nutrients are depleted. The data indicated that in addition to expected changes (e.g., energy conversion, protein turnover, translation, transcription, and DNA replication/repair) genes related to phage, stress response, carbohydrate flux, outer envelop, and iron homeostasis played important roles as *D. vulgaris* cells experienced electron donor depletion.

A better understanding of how cells respond to environmentally relevant conditions will improve methods of bioremediation by enabling control of field parameters that significantly affect bacterial biochemical capacity. Based upon microarray expression data, a predicted sensor protein was selected for characterization. The protein, DVU3152, showed up-expression in response to nitrate, nitrite, and stationary-phase, and the putative protein was predicted to be a sensory box histidine kinase. Therefore, a mutant was constructed for phenotypic characterization. The ability to respond to

changing conditions is important for cell survival and biochemical capability *in situ*, and our studies will help elucidate the mechanisms important that allow the utilization of *D. vulgaris* for bioremediation purposes. Our preliminary results suggest that the mutant is deficient in long-term survival and appears to be related to susceptibility to sulfide. These results are intriguing due to the fact that sulfate-reducers produce sulfide as a natural end-product of sulfate-reduction. However, very little is known about sulfide toxicity and/or sensitivity in sulfate-reducing bacteria. Genomic and transcriptomic analyses better enable the identification of new protein function, and the continued elucidation of function for DVU3152 is an example of the utility of genomics-based approaches for the characterizations of biological function.

Summary: Lactate and sulfate were used for our growth experiments, and lactate and sulfate are two of the major electron donor and acceptors for most *Desulfovibrio* spp., and this fact is exemplified by the fact that the annotated *D. vulgaris* genome has six lactate permeases and three possible sulfate permeases. The different lactate and sulfate permeases displayed different trends of expression as the cells transitioned between the growth phases. These results suggested that different permeases were used with respect to changing nutrient levels. For example, DVU3284 might be a low K_m lactate transporter that is up-expressed as lactate levels decline, and DVU0053 and DVU0279 may be low capacity, high affinity and high capacity, low affinity sulfate transporters, respectively. An alternative explanation might be that the sulfate permeases are regulated in a growth-rate dependent mechanism. A growth-rate dependent mechanism of regulation for the sulfate permeases could be explained by the fact that sulfate can be relatively abundant in most environments compared to electron donors and is the preferred electron acceptor for SRB. A major check point for growth control could be the presence and level of electron donor and not necessarily electron acceptor per se. Further work will be needed to determine the individual role(s) for the different transporters, and the environmental conditions under which each is important.

The results indicated that a subset of approximately 110 genes were uniquely up-expressed as the cells transitioned to stationary-phase. The rest of the genes up-expressed during stationary phase were also up-expressed during other stresses (i.e., NO_2, NaCl, pH, and heat) (unpublished results). The subset of up-expressed genes unique to stationary phase was mainly involved with DNA repair, nucleic acid metabolism, amino acid metabolism, and carbohydrate metabolism. Of the unique stationary genes that were up-expressed, 14 (13% of the total) were located on the mega-plasmid.

It should be noted that up to 17 genes (11% of megaplasmid ORFs) on the megaplasmid are predicted to play a role in carbohydrate metabolism, and total carbohydrate levels decreased approximately 2-fold when the cells transitioned to stationary-phase growth. During the transition, the putative isoamylase and glucan phosphorylase were up-expressed, and a putative glucan synthase and glucan branching enzyme were down-expressed. In addition, the one putative fbp (fructose-1,6-bisphosphatase) for *D. vulgaris* was down-expressed, and this result suggested a carbon flow in glycolysis from glucose towards pyruvate and not gluconeogenesis. Interestingly, the *D. vulgairs* genome has a putative fructose-2,6-biphosphatase (DVU3147), which is a unique characteristic for a

bacterium. DVU3147 displayed an upward trend in expression during the transition, and is located upstream from a putative glucotransferase in both *D. vulgaris* and *Desulfovibrio* G20. The fructose-2,6-biphosphatase is thought to be important in the regulation of hepatic carbohydrate metabolism, but the role in *Desulfovibrio* is not known.

When 101 genera with sequenced genomes were checked for the presence of a putative *rpo*S, only 21 had a predicted *rpo*S factor. These data suggested that rpoS is not a universal transcriptional factor for bacteria, and alternative mechanisms exist for the regulation of stasis response in different microorganisms. It is feasible that *D. vulgaris* has a factor that functions as an rpoS, but does not have significant sequence similarity to previously reported sigma factors. Further work will be needed to determine the control factor(s) in *D. vulgaris* involved in sensing environmental stimuli, and what cellular systems are required for cells to survive stasis. Our results indicated that in addition to expected changes (e.g., energy conversion, protein turnover, translation, transcription, and DNA replication/repair) that genes related to phage, carbohydrate flux, outer envelop, and iron homeostasis played a major role in the cellular response to nutrient deprivation under the tested growth conditions.

We continue to coordinate with the VIMSS:GTL project "Rapid deduction of stress response pathways in metal/radionuclide reducing bacteria" in order to facilitate data interpretation, minimize overlap, and maximize productivity. The microarrays (development and construction) supported by this project have been used by the GTL project for stress response experiments. The identification of stasis-induced genes and gene networks will provide fundamental information about the cellular processes needed for survival under pertinent field conditions (e.g., low-nutrients and slow-growth), and a comparative framework for additional stressors and environmental stimuli.

Publications

He, Z., L. Wu, M.W. Fields and J. Zhou. 2005. Comparison of microarrays with different probe sizes for monitoring gene expression. Appl. Environ. Microbiol. 71:5154-5162. Doi: 10.1128/AEM.71.9.5154–5162.2005

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Clark, M.E., Q. He, Z. He, E. Alm, K. Huang, T.C. Hazen, A. Arkin, J.D. Wall, J. Zhou, and M.W. Fields. 2006. Temporal transcriptomic analyses of *Desulfovibrio vulgaris* Hildenborough during electron donor depletion. Appl. Environ. Microbiol. 72:5578-5588. doi:10.1128/AEM.00284-06

Presentations

Z. He, Q. He, L.Wu, J.D. Wall, J. Zhou, M.W. Fields. 2004. Construction and evaluation of *D. vulgaris* whole-genome oligonucleotide microarrays and comparison of cells in exponential and stationary phase growth. American Society for Microbiology, 104th General Meeting, New Orleans, LA

X. Li, Z. He, C. W. Schadt, S. C. Chong, J. Liebich, T. J. Gentry, M. W. Fields and J. Zhou. 2004. Development of CommOligo to select unique and group specific probes for oligonucleotide microarrays using global alignment. American Society for Microbiology, 104th General Meeting, New Orleans, LA

M.W. Fields. 2004. Characterization of physiological responses of metal- and sulfate-reducing bacteria using genomics. presentatioin at Medical College of Ohio, Toledo, OH

Z. He, L. Wu, X. Li, M. W. Fields and J. Zhou. Empirical establishment of oligonucleotide probe design criteria. Abstract K-118, General Meeting of the American Society for Microbiology, Atlanta, GA

M. E. Clark, S. B. Thieman, J.D. Wall, M.L. Duley and M. W. Fields. Analysis of carbohydrate production in response to stasis in *Desulfovibrio vulgaris*. Abstract I-053, General Meeting of the American Society for Microbiology, Atlanta, GA

M.W. Fields, M.E. Clark, Z. He, Q. He, L. Wu, J.D. Wall, T.C. Hazen, J. Zhou Whole-Genome Expression Analysis of *Desulfovibrio vulgaris* Cells Throughout Exponential Phase into Stationary Phase Growth in a Defined Medium. Abstract K-120, General Meeting of the American Society for Microbiology, Atlanta, GA

M. W. Fields. Characterization of Diverse Microorganisms and Populations via Genomic Sequences and Physiological Traits. Invited presentation at the Michigan-ASM Branch Fall Meeting, University of Michigan-Dearborn, Dearborn, MI

Fields, M.W. Know Your Environment: From an Individual Microbe to Bacterial Communities. Invited presentation at Ohio Branch American Society for Microbiology Meeting. April, 2006.

Fields, M.W. Characterization of Diverse Microorganisms via Genomic Sequences and Physiological Traits. Invited presentation at University of Louisville, Department of Biology, October 2006.

Fields, M.W. Characterization of Microorganisms and Communities with Implications for Bioremediation. Invited presentation at Wright State University, Department of Biology, November 2006.

Fields, M.W. Characterization of Bacterial Biofilms in the Sulfate-Reducer, *Desulfovibrio vulgaris*, and in the Metal-Reducer, *Shewanella oneidensis*. Invited presentation at Indiana University-SE, Department of Natural Sciences, November 2006.