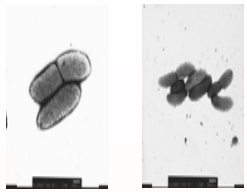
Construction and Evaluation of *Desulfovibrio vulgaris* Whole-Genome Oligonucleotide Microarrays

Z. He[1], Q. He[1], L. Wu[1], M.E. Clark[3], J.D. Wall[2], Jizhong Zhou[1] and Matthew W. Fields[1,3]

[1]Oak Ridge National Laboratory, Oak Ridge, TN; [2]University of Missouri, Columbia, MO; [3]Miami University, Oxford, OH

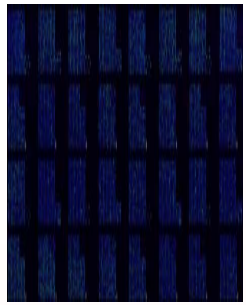
Abstract

Desulfovibrio vulgaris Hildenborough has been the focus of biochemical and physiological studies in the laboratory, and the metabolic versatility of this organism has been largely recognized, particularly the reduction of sulfate, fumarate, iron, uranium and chromium. In addition, a *Desulfovibrio* sp. has been shown to utilize uranium as the sole electron acceptor. *D. vulgaris* is a d-*Proteobacterium* with a genome size of 3.6 Mb and 3584 ORFs. The whole-genome microarrays of *D. vulgaris* have been constructed using 70mer oligonucleotides. All ORFs in the genome were represented with 3471 (97.1%) unique probes and 103 (2.9%) non-specific probes that may have cross-hybridization with other ORFs. In preparation for use of the experimental microarrays, artificial probes and targets were designed to assess specificity and sensitivity and identify optimal hybridization conditions for oligonucleotide microarrays. The results indicated that for 50mer and 70mer oligonucleotide arrays, hybridization at 45oC to 50ºC, washing at 37ºC and a wash time of 2.5 to 5 minutes obtained specific and strong hybridization signals. In order to evaluate the performance of the experimental microarrays, growth conditions were selected that were expected to give significant hybridization differences for different sets of genes. The initial evaluations were performed using *D. vulgaris* cells grown at logarithmic and stationary phases. Transcriptional analysis of *D. vulgaris* cells sampled during logarithmic phase growth indicated that 25% of annotated ORFs were up-regulated and 3% of annotated ORFs were down-regulated compared to stationary phase cells. The up-regulated genes included ORFs predicted to be involved with acyl chain biosynthesis, amino acid ABC transporter, translational initiation factors, and ribosomal proteins. In the stationary phase growth cells, the two most up-regulated ORFs (70-fold) were annotated as a carboxynorspermidine decarboxylase and a 2C-methyl-D-erythritol-2,4-cyclodiphosphate (MECDP) synthase. Spermidines are polyamines that are typically abundant in rapidly dividing cells and are essential growth factors in eukaryotic organisms. Polyamines are thought to stabilize DNA by the association of the amino groups with the phosphate residues of DNA and can also enhance tRNA and ribosome stability. The MECDP synthase enzyme is essential in *Escherichia coli* and participates in the nonmevalonate pathway of isoprenoid biosynthesis, a critical pathway present in some bacteria and apicomplexans but distinct from that used by mammals. Several of the highly up-regulated ORFs were annotated as conserved hypothetical proteins. Interestingly, an ORF that was predicted to contain a flocculin repeat domain was almost 9-fold up-regulated in stationary phase cells compared to logarithmically growing cells. The flocculin domain is commonly observed in fungi, and is thought to play a role during flocculation (non-sexual aggregation of single-cell microorganisms). These preliminary results have identified possible responses of *D. vulgaris* cells to stationary phase growth and suggest that polyamine production as well as cell aggregation and/or extracellular polymer production are responses of *D. vulgaris* during stationary phase. The initial microarray results indicate that the recently produced oligonucleotide microarrays are functional. We are currently optimizing growth conditions in order to culture *D. vulgaris* cells in the presence of uranium(VI) and to monitor whole-genome expression levels.

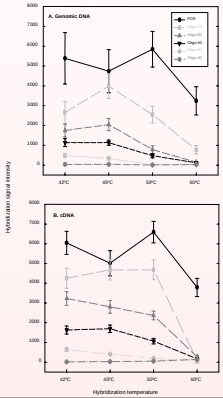


Construction of Whole-genome Oligonucleotide Microarrays of *Desulfovibrio vulgaris* Hildenborough

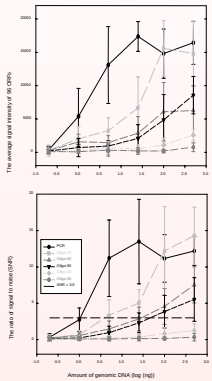
- All predicted ORFs represented by 3574 oligonucleotides
  3471 (97.1%) unique probes and 103 (2.9%) non-specific probes
- Oligonucleotide length = 70mer
- Negative controls: 10 oligo probes for 10 human genes and 10 oligos for 10 *Arabidopsis* genes
- Positive controls: different amounts of genomic DNA and probes for human genes and *Arabidopsis* genes with mRNA spiked in samples
- Each probe spotted in duplicate



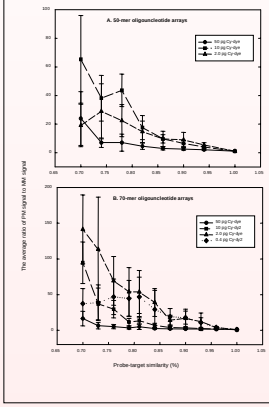
Comparison of Hybridization Signal Intensity to Probes of Different Length (30mer, 40mer, 50mer, 60mer, 70mer or PCR-product) with DNA or cDNA



Average Signal Intensity or SNR of 96 Probes of Different Length (30mer, 40mer, 50mer, 60mer, 70mer, PCR-product)

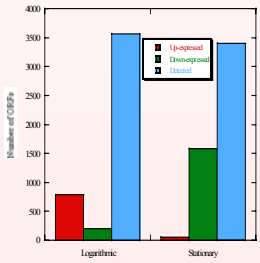


Relationship Between Probe-Target Similarity and Signal Intensity for 50mer and 70mer Probes



Comparisons of Designed Probe Information for *Desulfovibrio vulgaris* using different programs (probe length = 70-mer)

	ArrayOligoSelector	OligoArray2	OligoPicker
Designed Probes	3574 (99.7%)	1628 (45.4%)	3441 (96.0%)
Specific probes	3471 (97.1%)	1590 (44.4%)	3412 (95.2%)
Non-specific probes	103 (2.9%)	38 (1.1%)	29 (0.8%)
Rejected Sequences	10 (<0.3%)	1956 (54.6%)	143 (4.0%)
Total ORFs	3584	3584	3584
Run Time	~5 hrs	~6.5 hrs	~5 min



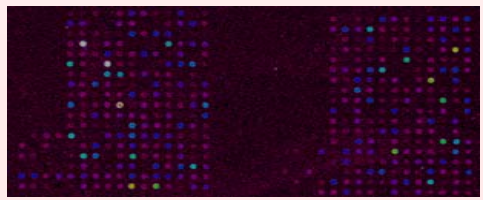
- 22% of ORFs were up-expressed in logarithmic phase cells (>4-fold)
- 6% of ORFs were down-expressed in logarithmic phase cells
- 1.6% of ORFs were up-expressed in stationary phase cells
- 44% were down-expressed in stationary phase cells
- Almost half of the 1.6% up-expressed stationary phase ORFs are hypothetical or conserved hypothetical ORFs

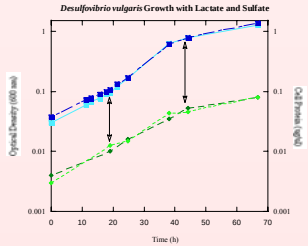
Up-Expressed ORFs During Logarithmic Phase

ORF ID	Fold Induction	Annotation
ORF00932	94X	Acyl Carrier Protein
ORF00247	83X	Hypothetical protein
ORF00933	73X	3-oxoacyl-(acyl carrier protein) reductase
ORF02862	61X	Amino acid ABC transporter
ORF04652	56X	Translation initiation factor IF-1
ORF03414	55X	Hypothetical protein
ORF05502	55X	tRNA pseudouridine synthase B
ORF01910	53X	Hypothetical protein
ORF02084	53X	Ribosomal protein S20
ORF05060	53X	Oxidoreductase/iron-sulfur cluster
ORF02652	52X	Translation initiation factor
ORF02650	50X	RNA-binding protein
ORF02534	45X	dnaK suppressor protein
ORF01909	44X	Ribosomal protein S21
ORF02917	43X	Outer membrane protein (putative)
ORF01887	42X	Conserved hypothetical protein
ORF01470	41X	Lipoprotein (putative)
ORF02323	41X	Recombination Protein
ORF04870	40X	DNA-binding response regulator
ORF05501	40X	Ribosomal protein S15

Up-Expressed ORFs During Stationary Phase

ORF ID	Fold Induction	Annotation
ORF05345	71X	Carboxynorspermidine decarboxylase
ORF01336	67X	2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase
ORF03029	25X	Small heat shock protein
ORF03920	25X	Phage shock protein A
ORF01584	24X	Conserved hypothetical
ORF05612	19X	Hypothetical protein
ORF04669	19X	Conserved hypothetical protein
ORF02390	15X	Chemotaxis protein
ORF05613	15X	Hypothetical protein
ORF00213	13X	Hypothetical protein
ORF01629	13X	σ^{54} factor family
ORF03769	12X	flagellar basal-body rod protein
ORF04474	12X	Hypothetical protein
ORF05010	12X	Conserved hypothetical protein
ORF05549	11X	hmc operon protein 4
ORF00550	11X	Lipoprotein (putative)
ORF01137	9X	Transcriptional regulator (putative LysR family)
ORF03074	9X	Flocculin repeat domain
ORF03611	7X	Electron transport complex protein (putative)
ORF02222	7X	Chaperonin





Conclusions:

- 70mer oligonucleotide probes perform in similar fashion to PCR-product based microarrays under the tested conditions
- The results indicated that for 50mer and 70mer oligonucleotide arrays, hybridization at 45ºC to 50ºC, washing at 37ºC and a wash time of 2.5 to 5 minutes gave specific and strong hybridization signals
- The arrays worked well when whole-genome expression patterns were compared for logarithmic and stationary phase cells
- Future work includes RT-PCR to confirm expression levels of selected ORFs
- Work is currently underway to determine the expression profiles of *D. vulgaris* cells when grown in the presence of sulfate-, chromium, and uranium as electron acceptors
- Identify specific systems of *D. vulgaris* that respond to increasing levels of nitrate and/or nitrite
- Further explore the responses of *D. vulgaris* cells to the stationary phase of population growth

OAK RIDGE NATIONAL LABORATORY
U. S. DEPARTMENT OF ENERGY

MIAMI UNIVERSITY