# Cellular Responses to Changing Conditions in *Desulfovibrio vulgaris* and *Shewanella oneidensis*

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## INTRODUCTION

*Desulfovibrio vulgaris* was cultivated in a defined medium and biomass was sampled over time for approximately 70 h to characterize the shifts in gene expression as cells transitioned from exponential to stationary phase growth during electron donor depletion. In the context of *in situ* bioremediation, nutrient scarcity and/or depletion may be a common obstacle encountered by microorganisms due to the oligotrophic nature of most groundwater and sediment environments. In addition to temporal transcriptomics; protein, carbohydrate, lactate, acetate, and sulfate levels were measured. The microarray data was used for statistical expression analyses, hierarchical cluster analysis, and promoter element prediction. As the cells transitioned from exponential to stationary-phase growth a majority of the down-expressed genes were involved in translation and transcription, and this trend continued in the remaining time points. Intracellular trafficking and secretion, ion transport, and coenzyme metabolism showed more up-expression compared to down-expression as the cells entered stationary phase. Interestingly, most phage-related genes were up-expressed at the onset of stationary-phase. This result suggested that nutrient depletion may signal lysogenic phage to become lytic, and may impact community dynamics and DNA transfer mechanisms of sulfate-reducing bacteria. The putative feoAB system (in addition to other putative iron-related genes) was significantly up-expressed, and suggested the possible importance of Fe2+ acquisition under reducing growth conditions for sulfate-reducing bacteria. A large subset of carbohydrate-related genes had altered gene expression, and the total carbohydrate levels declined during the growth phase transition. Interestingly, the *D. vulgaris* genome does not contain a putative *rpoS* gene, a common attribute of the δ-*Proteobacteria* genomes sequenced to date, and other putative rpo factors did not have significantly altered expression profiles. The elucidation of growth-phase dependent gene expression is essential for a general understanding of growth physiology that is also crucial for data interpretation of stress-responsive genes. In addition, 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, such as the changing ratios of electron donors and acceptors. Our results indicated that genes related to phage, internal carbon flow, outer envelop, and iron homeostasis played important roles as the cells experienced electron donor depletion.

*Shewanella oneidensis* MR-1, a Gram-negative facultative anaerobe, can utilize a wide array of alternative electron acceptors during anaerobic respiration, and the ability to reduce soluble forms of heavy metals to insoluble forms makes it a potential candidate for bioremediation studies. Understanding the physiological responses of *S. oneidensis* to environmental stresses (e.g., nutrients, oxygen) is important for the assessment of potential impacts on metal-reducing activity. Here we describe the physiological role of a presumptive signal transduction protein in *Shewanella oneidensis* MR-1. The predicted ORF (SO3389) encoded a GGDEF, EAL, and two PAS domains. The deduced amino acid sequence was not closely related to previously described proteins, but presumptive proteins with similar domain architectures were observed in metabolically diverse microorganisms. An in-frame, deletion mutant was constructed (ΔSO3389), and the mutant displayed an extended lag period (30 h) when transferred from aerobic to anaerobic medium. The mutant was also defective in motility, cytochrome content, and was drastically defective in biofilm formation. These pleiotropic phenotypes were observed with multiple growth substrates. During the transition from aerobic to anaerobic conditions, the mutant was deficient in three c-type cytochromes (57, 33, and 20 kDa). In addition, mutant biofilms produced less carbohydrate compared to wild-type cells. Bacterial motility was affected only in aerobic conditions, and this result suggested that SO3389 was involved in $O_2$ responses and was important for anoxic and biofilm growth.

## RESULTS Transcriptomic Responses to Electron Donor Depletion in *Desulfovibrio vulgaris*

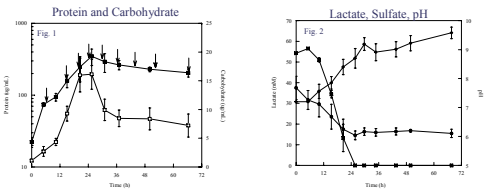


**Figure 1**. Protein (■) and carbohydrate (□) levels during growth of *D. vulgaris* cells with lactate (55 mM) and sulfate (35 mM). Arrows denote times at which biomass was removed for RNA extraction. **Figure 2**. Lactate (■), sulfate (●), and pH (◆) levels during growth of *D. vulgaris* with lactate (55 mM) and sulfate (35 mM).

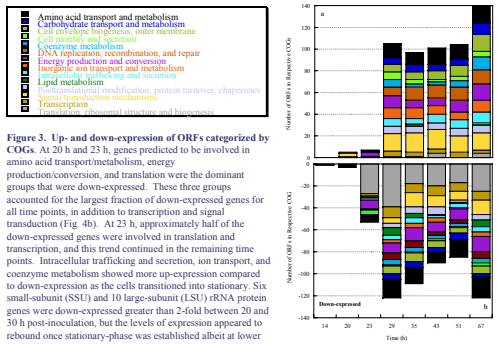


**Figure 3. Up- and down-expression of ORFs categorized by COGs**. At 20 h and 23 h, genes predicted to be involved in amino acid transport/metabolism, energy production/conversion, and translation were the dominant groups that were down-expressed. These three groups accounted for the largest fraction of down-expressed genes for all time points, in addition to transcription and signal transduction (Fig. 4b). At 23 h, approximately half of the down-expressed genes were involved in translation and transcription, and this trend continued in the remaining time points. Intracellular trafficking and secretion, ion transport, and coenzyme metabolism showed more up-expression compared to down-expression as the cells transitioned into stationary. Six small-subunit (SSU) and 10 large-subunit (LSU) rRNA protein genes were down-expressed greater than 2-fold between 20 and 30 h post-inoculation, but the levels of expression appeared to rebound once stationary-phase was established albeit at lower levels than observed for exponential-phase growth

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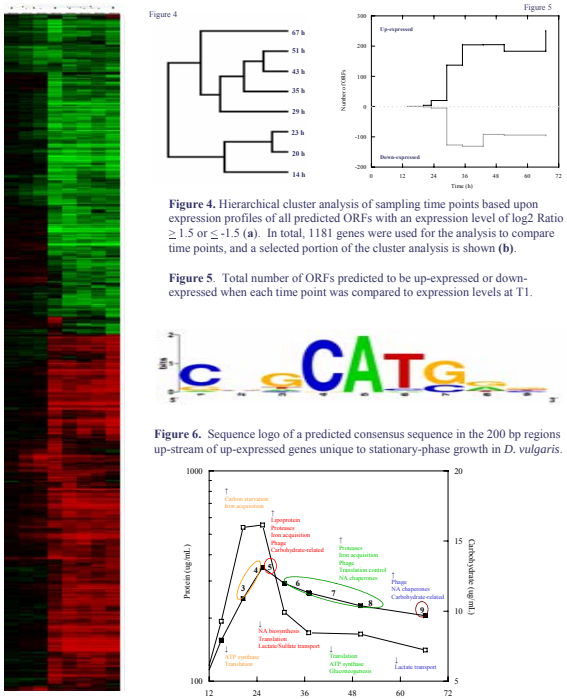


Figure 4




Figure 5




**Figure 4.** Hierarchical cluster analysis of sampling time points based upon expression profiles of all predicted ORFs with an expression level of log2 Ratio ≥ 1.5 or ≤ -1.5 (**a**). In total, 1181 genes were used for the analysis to compare time points, and a selected portion of the cluster analysis is shown (**b**).

**Figure 5**. Total number of ORFs predicted to be up-expressed or down-expressed when each time point was compared to expression levels at T1.



**Figure 6.** Sequence logo of a predicted consensus sequence in the 200 bp regions up-stream of up-expressed genes unique to stationary-phase growth in *D. vulgaris*.




**Figure 7**. General summary of significant up- and down-expressed genes at late-exponential (T3/T4), early stationary (T5), mid-stationary (T6/T7/T8), and late stationary phase (T9) growth).

**Some Major Conclusions from DVH Transcriptomic Study During e⁻ Donor Depletion**

- More ORFs were up-expressed during stationary phase than down-expressed
- In addition to expected changes (e.g., energy conversion, protein turnover, translation, transcription, and DNA replication/repair) genes related to : ***phage*** ***carbohydrate flux*** ***outer envelop*** ***iron homeostasis*** played a major role in the cellular response to nutrient deprivation under the tested growth conditions
- Stationary-phase response was coordinated without predicted rpoS and gene expression was not static during stationary-phase
- 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).
- A large subset of carbohydrate-related genes had altered gene expression, and the total carbohydrate levels declined during the growth phase transition

## RESULTS Deletion of predicted sensory-box ORF affects anoxic and biofilm growth in *S. oneidensis* MR-1

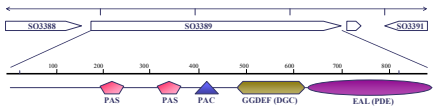



**Figure 1.** Genome region view and domain architecture of SO3389. SO3388 is annotated as an RNA helicase, SO3390 is annotated as a small (36 aa) hypothetical protein, and SO3391 is annotated as an ATP-dependent protease. The domains were predicted with SMART v4.0 (smart.embl-heidelberg.de/).

## RESULTS Deletion of predicted sensory-box ORF affects anoxic and biofilm growth in *S. oneidensis* MR-1

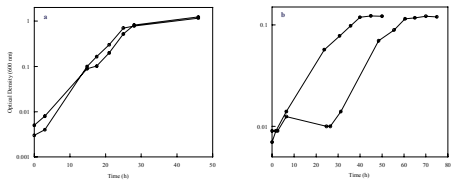
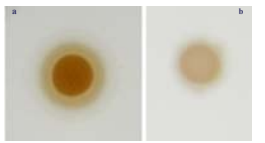



**Figure 2.** Growth curves of wild-type MR-1 and △SO3389 cells in aerobic (**a**) and anaerobic (**b**) defined, minimal medium with lactate or lactate and fumarate, respectively.

**Figure 3.** A colony of wild-type (**a**) or △SO3389 (**b**) cells on 0.3% motility agar inoculated from exponential phase aerobic cultures. SDS-PAGE gels of spheroplast (lanes 1 and 2) and periplasmic-shock (lanes 3 and 4) fractions of wild-type and △SO3389 cells stained with Coomassie blue (**c**) or cytochrome-c heme stain (**d**). Equal amounts of total protein for wild-type (lanes 1 and 3) and △SO3389 (lanes 2 and 4) were loaded per well, and molecular weight markers were as follows (kDa): 150, 100, 75, 50, 35, 25, and 15.



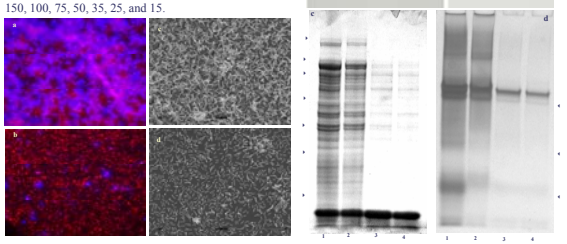




**Figure 4**. Epifluorescent microscopy of wild-type (**a**) and △SO3389 (**b**) biofilms grown in defined, minimal medium with lactate for 60 h. Acridine orange was used to stain cells (red) and calcofluor white was used to detect extracellular carbohydrate (blue). Scanning electron micrographs of wild-type (**c**) and △SO3389 (**d**) biofilms grown in defined, minimal medium with lactate for 20 h.

**Conclusions for the MR-1 Mutant**

- The mutant was deficient in:
  - transitions from aerobic to anoxic conditions
  - motility
  - c-type cytochrome content
  - biofilm formation
- Once the mutant entered exponential growth under anaerobic conditions, the growth rate was similar to wt
- Results suggested a role for a one-component signal transduction molecule in transitions from aerobic to anoxic growth and biofilm formation
- This is the first report of a multi-domain PAS protein involved in biofilm formation
- SEM and epifluorescent microscopy suggested that wt cells produced an exopolysaccharide matrix that was not observed in the mutant cells
- $O_2$ may be an important signal sensed by the protein, but further work is needed

## ACKNOWLEDGEMENT

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