ESPP Functional Genomics and Imaging Core: Cell wide analysis of Metal-Reducing Bacteria

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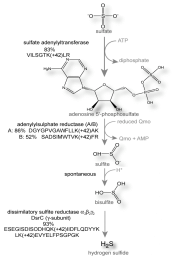
INTRODUCTION

A fundamental goal of the Environmental Stress Pathway Project (ESPP) of the Virtual Institute of Stress and survival (VIMSS) is a rigorous understanding of *Desulfovibrio vulgaris* Hildenborough physiology and its ability to survive in its environment. Such knowledge will be critical in discerning the biogeochemistry at metal contaminated sites, for bioremediation and natural attenuation for toxic metals. The Functional Genomics and Imaging Core (FGIC) focuses on the measurement of these responses at a cell wide level using systems biology approaches.

Progress in the last one year built upon our optimized pipeline for generating biomass for various functional genomics studies and utilized improved genetic methods. Numerous additional transcriptomics data sets were added to our compendium of stress response studies. These included peroxide stress, low oxygen stress, high and low pH stress and alteration of growth conditions (e.g presence of methionine, alternate electron donors etc). To understand how genotype and environment interact to determine the phenotype and fitness of an organism, a long-term evolution experiment was also conducted to examine the dynamics and adaptation of *D. vulgaris* under extended salt exposure. For many of these stresses iTRAQ based quantitative proteomics and CE-MS based metabolite studies were also conducted. Improved genetic methods were employed to create several critical knock out mutants (e.g. *echA*, *qmoABC and tatA*) and several were characterized via growth and transcriptomics studies. Progress was also made in extending transcriptomics analysis to examine alternate *D. vulgaris* physiological states such as in biofilms and growth in syntrophic co-culture with *Methanococcus maripaludis*. Methods to conduct iTRAQ proteomics and stable isotopomer (13C) based metabolic flux analysis were also developed for studying co-cultures. A novel FTICR-MS based method for a comparative 12C/13C based metabolite analysis is being developed and will enable a direct comparison of control cultures to experimental samples. Additionally we continued to collect cell wide data in *Shewanella oneidensis* and *Geobacter metallireducens* for comparative studies. Great progress was made in improving extraction and high throughput of metabolite studies. Metabolite extraction and CE-MS detection for several hundred metabolites can now be conducted for these non-model organisms using high resolution separation and high resolution mass spectrometric methods.

Continued studies to map cell wide responses have also emphasized the importance of changes that require orthologous measurements. With this in mind a novel protocol to monitor protein-protein interactions and redox state of the proteins has been developed. In an effort to supplement model development and elucidate intricacies of stress response cascades, comprehensive methods for identifying alternative regulatory mechanisms such as small non-coding RNAs are also underway. To optimize the use of the large amounts of data being collected, several data mining efforts were initiated. For example, iTRAQ data sets from the multiple stress response studies were mined for potential post translational modifications and confirmation of hypothetical proteins while ^{13}C flux data were used to confirm gene annotation and assess missing steps in metabolic pathways. Work in underway in collaboration with the computational core to set up searchable databases of our proteomics and metabolite data also.

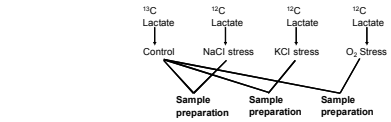
Mining proteomics data for PTMs in *D. vulgaris*

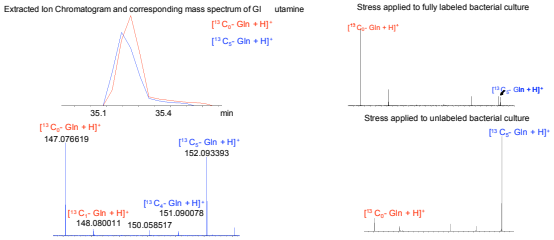


Advances in mass spectrometric data analysis now allow mining of large proteomics datasets for the presence of post translational modifications (PTMs). Although PTMs are a critical aspect of cellular activity, such information eludes cell wide studies conducted at the transcript level. We analyzed several mass spectrometric datasets from earlier D. vulgaris studies. Our searches of the raw spectra led us to discover several post translationally modified peptides in *D. vulgaris*. Of these, several peptides containing a lysine with a +42 Dalton (Da) modification were found reproducibly across all datasets. Both acetylation and trimethylation have the same nominal +42 Da mass, and are therefore candidates for this modification. Surprisingly, these modified peptides predominantly mapped to proteins involved in sulfate respiration and maybe important in this process. Other highly expressed proteins in *D. vulgaris*, such as enzymes involved in electron transport and other central metabolic processes, did not contain this modification.

Using metabolomics to understand cellular response

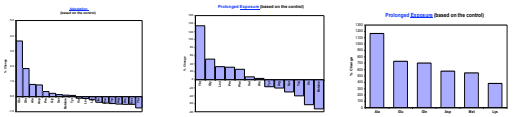
Quantitative Analysis of the Microbial Metabolome via Isotope labeling



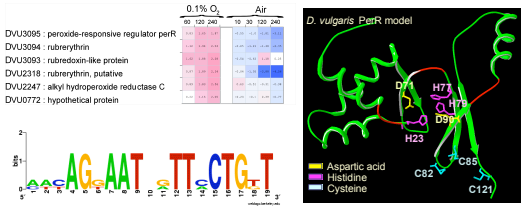


The $^{12}C/^{13}C$ labeling strategy outlined above allows state of the art mass spectrometry workflows to be applied to obtaining quantitative measures of metabolic changes. In this strategy isotopic dilution strategy reliably quantifies the metabolites. Further, use of computational methods enables identification of unknown metabolites. Shown below is a more classical method to obtain changes in metabolite levels using internal standards.

The percent change in amino acid levels as a result of DvH adaptation to 250mM NaCl or prolonged exposure of *D. vulgaris* to 250mM NaCl



Follow-up on cell wide studies



PerR is a global gene regulator in *D. vulgaris* and is predicted to regulate several oxidative response genes. The *D. vulgaris* PerR (208612) is very homologous to the *B. subtilis* PerR any operate via the same mechanism. Here we show the *D. vulgaris* was modeled on the *P. aeruginosa* Fur structure (1mzbA) using SwissModel. Also shown is the sequence consensus predicted to be regulated by PerR. Based on these prediction and the experimental data for mRNA changes during oxidative stress, we can confirm and extend the regulon prediction of PerR in *D. vulgaris*.

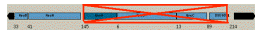
Genetic and gene regulation in *D. vulgaris*

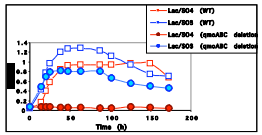
D. vulgaris deletion constructs

qmoABC

The *qmoABC* genes are located downstream to the known sulfate reduction genes, *apsBA*, in numerous bacteria. The *qmoABC* are predicted to form a transmembrane complex to deliver electrons to APS, Adenylylsulfate reductase.

ΔqmoABC

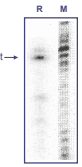




Gene deleted	Phenotype observed
Cytochrome c_3 (*cycA*)	Defects in energy metabolism
Glycogen synthase, Glycogen phosphorylase (*glgA, glgP*)	None yet observed (WT)
Lactate dehydrogenase (*ldh*)	None yet observed (WT)
Putative methionine biosynthesis gene (putative *metW*)	Not a methionine auxotroph (WT)
Uncharacterized dehydrogenase (*nox*)	Multiple growth differences
Polyribonucleotide phosphorylase (*pnp*)	Reduced growth
Quinone-interacting membrane-bound oxidoreductase (*qmoABC*)	Unable to reduce sulfate
Twin-arginine translocase, subunit A (*tatA*)	No growth on formate

Identification of a sRNA in *D. vulgaris*

- Dv-sRNA2 was identified from randomly cloning the small RNA fraction.
- Northern analysis of *D. vulgaris* total RNA under normal LS4D growth conditions indicated that Dv-sRNA2 is transcribed.
- Based on sequence analysis, the sRNA appears to be regulated by sigma 54 and binds to the RBS of DVU0678 (unknown protein) in an antisense fashion, thus blocking translation of DVU0678.
- Work is currently underway to determine the effect over-expressing Dv-sRNA2 has on the cell.



R: 10 µg total RNA
M: Marker

Continued environmentally relevant cell wide studies

The functional genomics core of the ESPP project continues to focus on cells wide studies in *D. vulgaris*, such as microarray analysis, to better understand its physiology in environmentally relevant conditions.

In addition to studies in oxidative stress, pH stress, heavy metal stress etc, the past year has seen much effort directed towards developing methods to study other physiological states (stationary state, biofilm culture) and also to study the effect of co-culture growth on this bacteria.

Availability of mutants and data mining from cell wide studies, such the isotopomer based pathway flux analysis, are enabling experimental validation of the annotated genome and whole filling of missing pathways.

Studies in gene regulation and signal sensing in *D. vulgaris* will improve our understanding of not only this bacteria but will improve our knowledge of bacterial physiology in general.

ACKNOWLEDGEMENT

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