Characterization of a *Desulfovibrio vulgaris* Hildenborough Mutant Strain Lacking the Ferric Iron Uptake Regulator (*fur*) Gene

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INTRODUCTION

Analysis of wild-type *Desulfovibrio vulgaris* Hildenborough under a variety of stress conditions suggests a role for the ferric uptake regulator (FUR) in the general stress response by this organism. A mutant *D. vulgaris* strain lacking the *fur* gene has previously been constructed and analyzed using physiological and molecular biology techniques. The mutant strain, designated JW707, was analyzed under iron-replete and iron-depeleted conditions using whole-genome microarray techniques to complement the previous analyses. JW707 cultures grown to mid-log phase with 60 μM Fe showed upregulation of 51 genes (z ≥ 2). These genes included a ferrous iron transport operon (*feoAB*), a TonB-dependent ABC transport operon (including *tolQR*), a putative pepsidase/ABC transporter operon and an uncharacterized hypothetical protein-encoding gene (DVU2681) also shown to be induced under heat shock and nitrite stress conditions. These results are consistent with previously conducted Northern analyses and computational predictions of the *D. vulgaris* FUR regulon. Similar results were observed for cells grown under iron-depleted conditions (5 μM Fe). In addition, elements of the Trp and Met biosynthetic operons and the Zur-dependent zinc transport operon were upregulated and elements of the flagellar apparatus were down-regulated in the mutant. Also analyzed was a *Dvu* strain lacking the gene encoding the FUR paralog PerR, a transcriptional regulator of the oxidative stress response.

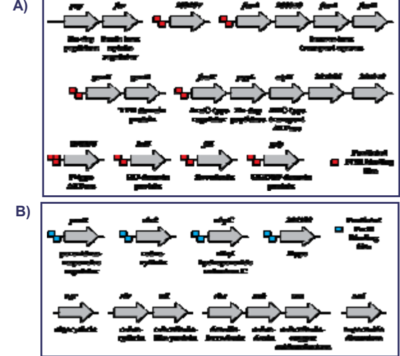
Experimental Conditions

Cell culture and treatment: wt and JW707 *fur* *D. vulgaris* cells were grown at the LS medium with 60 μM or 5 μM iron. wt and JW708 *perR*⁻ *D. vulgaris* cells were grown on standard LS medium. Cells from each culture were harvested at midlog and early stationary pahse.

D. vulgaris oligonucleotide array: 70mer oligonucleotide arrays that containing all ORFs were constructed as described (He et al., in press).

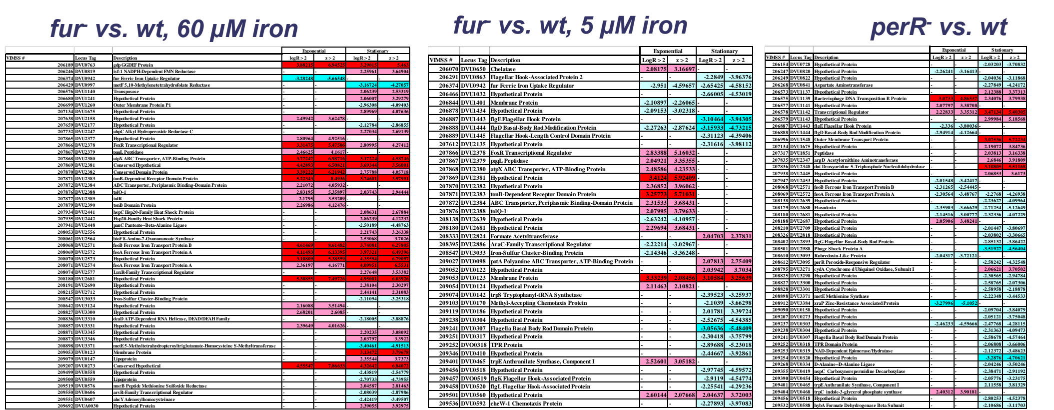
Target preparation, labeling and array hybridization: Total cellular RNA was isolated and purified using TRIzolTM Reagent, and then labeled with Cy5 dye. Genomic DNA was isolated and purified from *D. vulgaris* as described previously (Zhou et al., 1996), and then labeled with Cy3 dye. The labeled RNA and genomic DNA were co-hybridized to the array at 45oC with 50% formamide for 16 hrs in the dark. Image and data analysis were the same as described previously (Chhabra et al., 2006; Mukhopadhyay et al., in press).

Predicted FUR Regulon of *D. vulgaris*

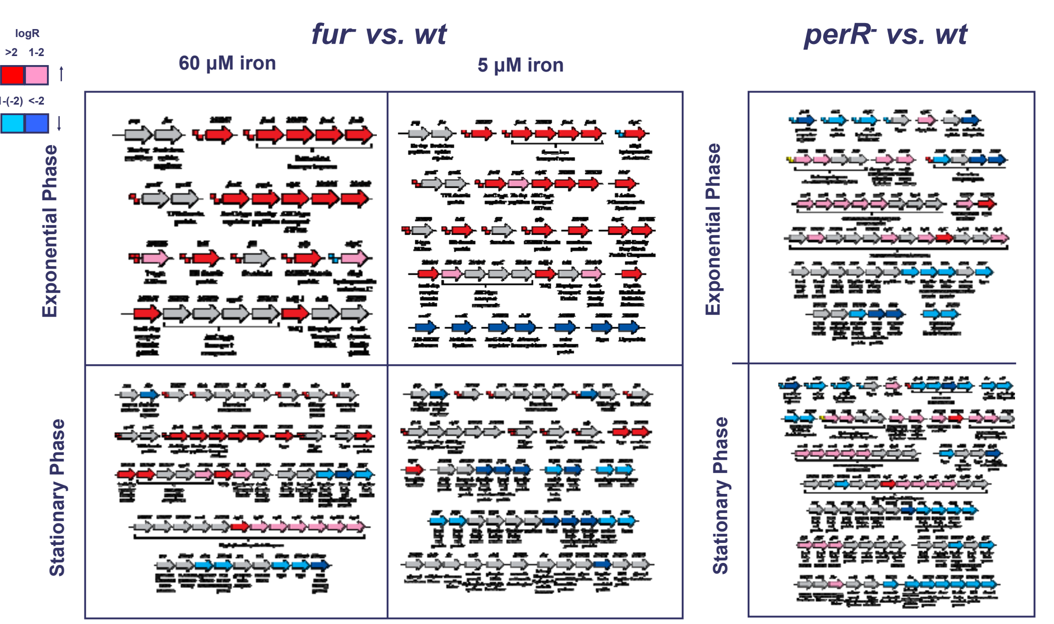


The computationally predicted FUR (A) and PerR (B) regulons defined by Rodionov et al., 2004. The PerR regulon figure (B) also includes genes encoding known oxygen defense proteins employed by *D. vulgaris*, but it is not currently known if these genes are regulated by PerR binding.

Whole-Genome Transcriptomic Analysis of the *D. vulgaris fur* and *perR*⁻ Strains



Operons Affected by FUR and PerR (z > 2)



The microarray results for the *fur* are consistent with the computationally predicted regulons and with previously described Northern hybridization experiments. These results suggest that the regulons may be more complex than previously predicted. A degree of overlap appears to exists between the FUR, PerR and ZUR regulons.

Role of Methionine in *D. vulgaris* Stress Response

Observations:
- Expression of *metE* decreases in *fur* strain in stationary phase at 5 μM iron
- Expression of *metE* decreases in *perR*⁻ strain in stationary phase
- Stress conditions can induce Met auxotrophy in *E. coli* by inactivation of *metE* which in turns results in accumulation of toxic levels of homocysteine

Questions:
- Is the *metE* phenotype caused by stress or the transition to stationary phase?
- Is this response related to Fur?

Hypotheses
- Inactivation of *metE* during stress conditions limits growth of *D. vulgaris* by inducing Met auxotrophy
- This effect is accompanied by the accumulation of homocysteine in the cell
- JW707 strain grown in iron replete medium supplemented with Met will show increased growth yields compared to standard medium
- *metE*⁻ strain will show similar expression profile as stressed cells

Role of Tryptophan in *D. vulgaris* Stress Response

Observations:
- Expression of Trp biosynthesis genes are increased in JW707 at 5 μM iron
- Expression of Trp biosynthesis genes are increased in *perR*⁻ strain during exponential and stationary phases
- Expression of Trp biosynthesis genes are increased in cells stressed with nitrate or NaCl
- Several plant and bacterial species employ secondary metabolites derived from Trp in the oxidative stress response

Questions:
- Is Trp biosynthesis part of the general stress response of *D. vulgaris*?

Hypotheses
- *D. vulgaris* responds to redox stress by producing secondary metabolites from Trp to act as antioxidants
- A Trp auxotroph will be more sensitive to oxidative stress than the wild type

Expression of Met and Trp Biosynthesis Genes of Mutant Strains Compared to Other ESPP *Dvu* Microarray Experiments

Future Work

- Transcriptomics analysis of mutants strains under stress conditions
- Distinguish between responses dependent and independent of FUR/PerR
- Analysis of roles of Trp and Met biosynthesis enzymes in general and specific stress responses

References

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See also posters I-006, I-012, I-017 and Q-238

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