EXAMINATION OF METABOLIC AND REGULATORY NETWORKS OF *DESULFOVIBRIO* SPECIES

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Abstract

The sulfate-reducing bacteria are a morphologically diverse group of organisms characterized by the ability to couple the enzymatic reduction of sulfate to energy production and growth. This metabolic activity has profound economic and environmental consequences such as the corrosion of metal structures and the souring of petroleum reserves. The sulfate-reducing bacteria are also among a select group of organisms that may be used as tools for the bioremediation of toxic heavy metal contaminants from the environment. To understand the mechanisms through which these bacteria impact our environment both positively and negatively, genomic studies have been undertaken to predict the metabolic and regulatory networks of two species of the genus *Desulfovibrio*. Studies have focused on the elucidation of carbon metabolic pathways, the role of CRP-FNR proteins in the regulation of *Desulfovibrio* metabolic pathways, and the prediction of global regulatory networks using bioinformatics techniques.

Surprisingly, several hexose metabolic genes were found despite the fact that biochemical evidence suggests that these bacteria do not use hexose sugars as growth substrates. This physiological paradox was explored. Secondary pathways for the metabolism of galactose and the synthesis of α,α-trehalose were observed in *Desulfovibrio desulfuricans* G20 but not *Desulfovibrio vulgaris* Hildenborough. Physiological experiments showed that despite the presence of a complete set of galactose metabolism genes, *Dv. desulfuricans* was unable to utilize galactose as the sole carbon source for growth. Growth experiments using [^{14}C]-labeled galactose in the presence of lactate suggested that galactose was incorporated into the cell. This result coupled with the published observation that the extracellular polymeric substances (EPS) of *Dv. desulfuricans* strains contained detectable levels of galactose suggests that metabolism of galactose occurs for the purposes of production of EPS and possibly lipopolysaccharide (LPS).

To explore the possible hierarchical regulation of substrate utilization, global regulators responding to redox signals were sought. Sequencing revealed multiple orthologs of the CRP-FNR genes in *Desulfovibrio*. A mutant strain of *Dv. vulgaris* was constructed that was interrupted in a gene encoding a putative CRP-FNR protein. A phenotypic analysis of the mutant strain showed no significant differences in the growth rates and growth yields of the cells compared to wild type when grown on lactate-sulfate, pyruvate-sulfate (respiration), pyruvate alone (fermentation) or formate-sulfate. However, the mutant was shown to be impaired in growth on ethanol-sulfate compared to wild type, suggesting the involvement of this protein in either the cellular ability to use ethanol or maintain cellular integrity.

A computational analysis of the promoter regions of putative transcription units of *Desulfovibrio* revealed possible regulatory protein binding motifs homologous to the *E. coli* CRP and GalR binding sites as well as a motif common to genes encoding phosphate homeostasis proteins. Further examination revealed a set of statistically significant motifs not immediately identified as *E. coli* homologs. This set of motifs may represent unique regulatory motifs of *Desulfovibrio*.