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In Silico Modeling of Geobacter Species.
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This project employed a combination of *in silico* modeling and physiological studies to begin the construction of models that could predict the activity of *Geobacter* species under different environmental conditions. A major accomplishment of the project was the development of the first genome-based models of organisms known environmental relevance. This included the modeling of two *Geobacter* species and two species of *Pelobacter*. Construction of these models required increased sophistication in the annotation of the original draft genomes as well as collection of physiological data on growth yields, cell composition, and metabolic reactions. Biochemical studies were conducted to determine whether proposed enzymatic reactions were in fact expressed.

During this process we developed an Automodel Pipeline process to accelerate future model development of other environmentally relevant organisms by using bioinformatics techniques to leverage predicted protein sequences and the Genomatica database containing a collection of well-curated metabolic models. The Automodel Pipeline was also used for iterative updating of the primary *Geobacter* model of *G. sulfurreducens* to expand metabolic functions or to add alternative pathways. Although each iteration of the model does not lead to another publication, it is an invaluable resource for hypothesis development and evaluation of experimental data.

In order to develop a more accurate *G. sulfurreducens* model, a series of physiological studies that could be analyzed in the context of the model were carried out. For example, previous field trials of *in situ* uranium bioremediation demonstrated that *Geobacter* species face an excess of electron donor and a limitation of electron acceptor near the point of acetate injection into the groundwater. Therefore, a model-based analysis of electron acceptor limitation physiology was conducted and model predictions were compared with growth observed in chemostats. Iterative studies resulted in the model accurately predicting acetate oxidation and electron acceptor reduction. The model also predicted that *G. sulfurreducens* must release hydrogen under electron-accepting conditions in order to maintain charge and electron balance. This prediction was borne out by subsequent hydrogen measurements. Furthermore, changes in gene expression were consistent with model predictions of flux changes around central metabolism.

The model revealed multiple redundant pathways in central metabolism suggesting an apparent versatility unusual in microbial metabolism. The computational analysis led to the identification of 32 reactions that participated in eight sets of redundant pathways. The computational results guided the design of strains with mutations in key reactions to elucidate the role of the alternate pathways and obtain information on their physiological function. A total of seven strains with mutations in genes encoding five metabolic reactions were constructed and their phenotypes analyzed in 12 different environments. This analysis revealed several interesting insights on the role of the apparent redundant pathways.

^{13}C labeling approaches were developed for further elucidation of metabolic pathways with model-driven interpretation. For example, the model was used to calculate the optimal acetate ^{13}C labeling ratio for distinguishing flux through various pathways based on amino acid isotopomer distributions. With this method it was possible to elucidate the pathways for amino acid biosynthesis. Surprisingly, the labeling pattern of isoleucine deviated significantly from what was predicted by the metabolic reconstruction. Detailed analysis of the labeling patterns with the model led to the discovery that there are two pathways for leucine biosynthesis, including a novel citramalate pathway that was subsequently confirmed with biochemical analysis.

In summary, the combined computational and experimental studies have been instrumental in further characterizing the central metabolism of members of the *Geobacteraceae*. Furthermore, the methods developed in these studies provide a strategy for the genome-based study of the physiology of other understudied, but environmentally significant organisms.

Publications

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Segura, D., R. Mahadevan, K. Juárez, and D.R. Lovley. 2008. Computational and experimental analysis of redundancy in the central metabolism of *Geobacter sulfurreducens* . PLOS Computational Biology (in press).

Mahadevan, R. and D. R. Lovley. 2008. The degree of redundancy in metabolic genes is linked to mode of metabolism. Biophysical Journal (in press).

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Mahadevan R, Bond DR, Butler JE, Esteve-Núñez A, Coppi MV, Palsson BO, Schilling CH, Lovley DR (2006) Characterization of Metabolism in the Fe(III)-Reducing Organism *Geobacter sulfurreducens* by Constraint-Based Modeling. *Appl Environ Microbiol* 72 **(2)**:1558-68.

Leang C, Coppi MV, Lovley DR. (2003) OmcB, a *c*-Type Polyheme Cytochrome, Involved in Fe(III) Reduction in *Geobacter sulfurreducens*. *J Bacteriol* 185 **(7)**:2096-2103.

Four other manuscripts that were started under this project will be completed soon.