Pathway of Fermentative H2 Production by Sulfate-reducing Bacteria

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Project Goals: The abundant soil anaerobes, the sulfate-reducing bacteria (SRB) of the genus *Desulfovibrio*, produce H_2 from organic acids. These apparently simple pathways have yet to be clearly established and their elucidation forms the goals for our research efforts. Information obtained may facilitate the exploitation of other anaerobes not yet readily amenable for examination by molecular tools and will be useful in consideration of practical applications. Energy metabolism, flux through the pathways, and regulation are likely to be limiting factors in channeling reductant for H_2 production, the process which we are examining. Our research has focused on: (1) the determination of the path of electrons during pyruvate fermentation growth in order to identify possible electron sinks, (2) confirmation of the enzymes that are essential during pyruvate fermentation, and (3) determination of the role of the pyruvate enzymes during lactate respiration.

Abstract:

Strains of *Desulfovibrio* can ferment organic acids in the absence of additional terminal electron acceptors and produce rather large amounts of H_2. We believe a study of the limitations to H_2 production in our model organism, *Desulfovibrio alaskensis* G20 (Hauser et al, 2011) may be informative to decipher the flow of electrons in those organisms chosen for industrial application for H_2 production.

We proposed to tease apart the contribution of fermentation to the respiratory energy budget to determine the dependence of the bacterium on this process. In studying growth, we have realized an important role for the utilization of fumarate via the fumarate reductase complex (Dde_1258 – Dde_1256) during various growth modes. *D. alaskensis* G20 is able to grow robustly on fumarate through disproportionation after adaptation when subcultured from cells grown on lactate/sulfate. However, growth on fumarate is inhibited by formate, lactate, pyruvate, H_2, or CO_2 indicating that the use of fumarate by the cells may be a secondary or tertiary option as an electron acceptor. Interestingly, proteomic analysis reveals that even during

growth on lactate/sulfate or by pyruvate fermentation, the proteins of the fumarate reductase complex are expressed at relatively abundant levels. Yet, a very small proportion of reductant appears as succinate in these culture conditions.

Mutant analysis of the genesencoding the fumarate reductase complex, the quinone-reducing complex, and the type-1 tetraheme cytochrome c_3 indicate possible components playing a role in the flow of electrons, as all are unable to grow on fumarate. In addition, several mutants lacking formate dehydrogenase isozymes have an impaired growth phenotype on fumarate. Microarray, proteomic, and metabolite analysis is currently underway to determine changes that may be critical to robust growth of *D. alaskensis* G20 by disproportionation of this substrate and in the channeling of reductant for H_2 production.

References and Publications:

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2. Keller, K. L., and J. D. Wall. (2011) Genetics and molecular biology of the electron flow for sulfate respiration in *Desulfovibrio*. *Front Microbiol.* **2:**1-15.

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