

II.K.6 Genes Needed For H₂ Production by Sulfate Reducing Bacteria

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Objectives

Research objectives are to study genes and proteins within *Desulfovibrio* strain G20 that are involved in syntrophic interactions in coculture with a Syntrophic butyrate degrading bacterium and a during lactate oxidation in coculture with a hydrogenotrophic methanogen. Specifically, this has involved screening mutants for loss of ability to grow in coculture in order to identify genes in *Desulfovibrio* needed for syntrophic growth. Mutants, mutated genes and the proteins encoded by these genes are being characterized to determine their specific function during syntrophic growth. Characterization involves a determination of the effect of the mutation on growth characteristics as well as the properties of the protein encoded by that gene.

Technical Barriers

- Identify key enzymes involved in H₂ production by *Desulfovibrio*.
- Characterize these enzymes to determine their role in H₂ production.

Abstract

In order to identify proteins involved in H₂ metabolism in *Desulfovibrio desulfuricans* G20, transposon insertion mutants deficient in syntrophic growth were identified and are being characterized. A screening done with G20 in a hydrogen uptake role in coculture with *Syntrophomonas wolfeii* found that mutants for the *flhA* flagellar biosynthesis gene, the *fliF* flagellar ring gene, and the *pilA* pilus assembly gene were unable to grow, suggesting a role for chemotaxis or other flagellar function in syntrophy. Microscopy showed the *flhA* mutant to lack flagella and both flagella mutants and the pilus mutant produced considerably less biofilm than the parent strain. Recent experiments suggest that the flagella mutants grow more slowly on H₂, perhaps indicating a need to motility during H₂ growth in laboratory

cultures. We have also recently identified mutants in a membrane protein annotated as Rnf. This protein appears to be involved in H₂ uptake as G20 mutants will not grow on H₂ or formate. We are currently identifying the specific mechanisms by which the above proteins allow the cell to grow with H₂.

The membranes of several mutants of *Desulfovibrio vulgaris* including a *qmo* (quinone membrane oxidoreductase) mutant were extracted and separated on Blue Native gels to identify large protein complexes. The *qmo* mutant was found to be missing several bands compared to the parental strain. These bands were identified to be the *qmo* protein complex as well as a complex of the *qmo* and APS reductase, suggesting that these two proteins operate as a multiprotein complex during H₂ oxidation. Affinity tagged genes are being used to verify this phenomenon as well as to identify other proteins that may be involved in this electron transport complex.

Progress Report

Genes involved in syntrophic growth. A number of genes have been identified which appear to be required for syntrophic growth of *Desulfovibrio* as the H₂ user with *Syntrophomonas wolfeii*. Some characterization experiments have been done and results are presented below (table 1). Results show that both the presence of flagella as well as the Rnf protein play a role in H₂ uptake. Rnf has been characterized in *Rhodobacter capsulatus* and shown to be involved in nitrogen fixation (1). It is a large membrane spanning protein with homology to energy conserving oxidoreductases in many other bacteria. Although some species of *Desulfovibrio* contain genes for nitrogen fixation, strain G20 does not, suggesting that Rnf plays a different role. The mechanisms for disruption of H₂ metabolism will be investigated.

Membrane Protein Complexes in *Desulfovibrio*.

Several mutants were grown in culture and their membranes were isolated and run on Blue Native gels to determine whether they formed large complexes. Gels run with a mutant in the QMO complex had three absent bands in comparison to the parental strain (Fig. 1). Bands were excised and proteins were identified by mass spectroscopy. One of the bands (C) appears to be the *qmo* complex while another band (B) appears to be a *qmo* complex containing both QMO and APS reductase. This complex has been speculated to operate together for the reduction of sulfate (APS) to sulfite as both QMO genes and APS reductase genes are located adjacent to each other on the chromosome (2). As well, the QMO mutant will grow on sulfite but not on sulfate (3). This is the first evidence showing a physical interaction between these proteins. Affinity tagged strains have been produced and in

TABLE 1. Growth and biofilm formation by pure cultures of some syntrophy mutants. Results shown that mutations in flagellar function and pilus function affect both biofilm formation and growth on H₂. As well, rnf mutants do not grow on H₂. ND-not determined.

Gene number	Gene Name	Proposed Function	H ₂	Growth Formate	Pyruvate	Biofilm Formation
Dde_0380	flhA	Flagella Biosynthesis	++	+++	++++	+
Dde_2933	qrc	Hydrogen metabolism	-	-	++	ND
Dde_0353	fliF	Flagellar Ring Protein	++	+++	++++	+
Dde2365	tadC	Pilus assembly prot.	++	+++	++++	++
Dde_0585	rnfA	Electron Flow	-	-	++++	ND
Dde_0582	rnfD	Electron Flow	-	-	++++	ND
Parent			+++	+++	++++	+++

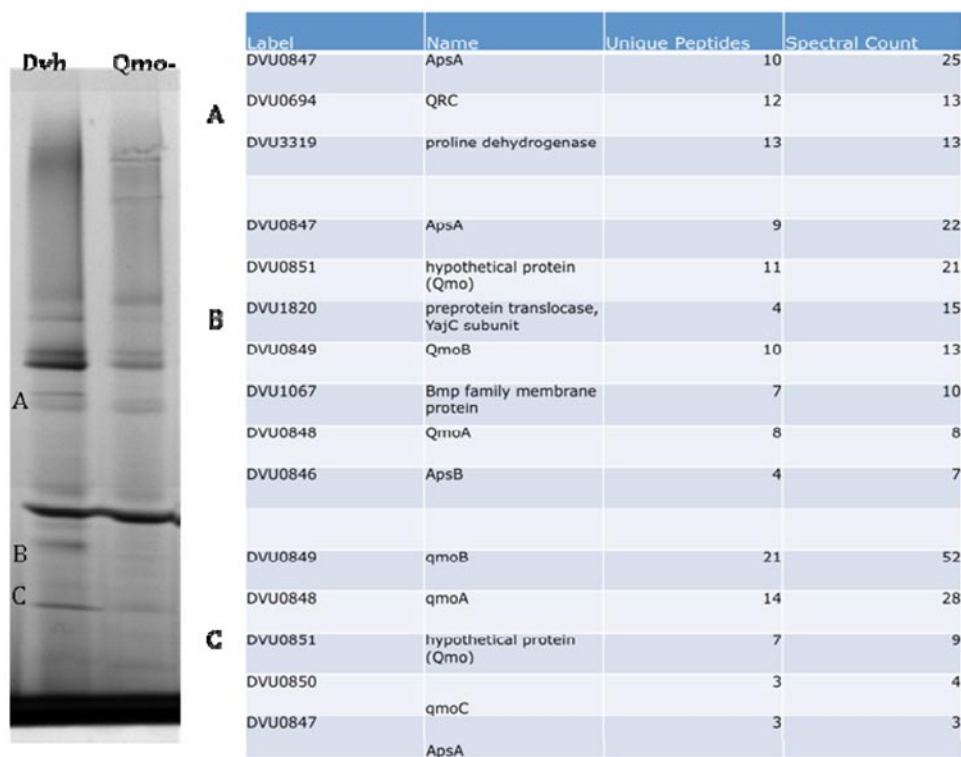


FIGURE 1. Blue native gel and peptide identification data for QMO mutant and WT.

the presence of detergent, both QMO and APS reductase have been purified to homogeneity from cell extracts of strain G20. These enzymes are expected to be present in complexes with each other as shown in figure 1. SDS gel banding patterns of each of the purified enzymes are identical suggesting that they all contain the same set of proteins as a complex. However, to be certain we are in the process of identifying protein bands from SDS gels using mass spectroscopy. This will prove the association of QMO reductase and determine whether any other proteins are associated with this complex.

Future Directions

Determination of the role of syntrophy genes. RNF appears to play a role in the syntrophic interaction. The inability of G20 rnf mutants to grow in the presence of H₂ may be the reason for its inability to grow syntrophically. Rnf is a large membrane spanning protein complex that couples electron transfer to energy transduction (1). It is thought to also involve quinone reduction. The growth of rnf mutants will be further investigated to determine whether Rnf may interact with QMO or with DSR directly

of perhaps whether it plays a more indirect role in H₂ uptake. This is the first study that I am aware of that implicates Rnf in H₂ metabolism by *Desulfovibrio*. A group of three flagella and pilus genes have also been shown to be involved in syntrophy. The specific role that these genes play will be investigated. Through growth experiments, chemotaxis studies as well as experiments looking at the physical interaction between the two members of the syntrophic consortium

Membrane Protein Complexes in Desulfovibrio. This work will build on the above results showing the presence of respiratory membrane complexes in strain G20. Our recent work has demonstrated a role for a protein now referred to as Qrc (Mop) in the uptake of H₂ by *Desulfovibrio* G20. Qrc has recently also been purified and shown to interact with both tetraheme cytochrome C3 in the periplasm and menaquinone (most likely in the membrane) (4). It is therefore possible that Qrc interacts directly with QMO for the oxidation of H₂ coupled to the reduction of adenosine phosphosulfate (APS). We will test this hypothesis by reconstituting purified enzymes tetraheme cytochrome C3, Qrc, QMO, APS reductase available with affinity tags along with menaquinone to determine whether this process can occur in vitro.

Transcriptomic Analysis of Syntrophic Cocultures. A number of genes have been identified that are involved in syntrophic interactions, based on growth assays. In the original proposal, we described experiments using microarrays to validate the role of these genes in syntrophy. New and more powerful technologies are available and the most promising of these is RNA-seq. This technique involves extracting mRNA from active cultures and sequencing the RNA using Illumina technology. This technique is advantageous over microarrays in that there is no up front cost and effort and information is not only obtained on the amount of each transcript present, but the complete sequence of each transcript is also obtained providing information on operon structure, etc. Pure cultures and syntrophic co-cultures will be grown to log phase, cells will be harvested and transcript abundance will be determined. We will compare cocultures of *Syntrophomonas wolfei*, *Syntrophus aciditrophicus* and *Desulfovibrio* G20 grown with *Methanospirillum hungatei*. Pure cultures will also be tested. In this way, comparisons can be made and genes expressed only in co-cultures can be compared between cocultures to identify those with a direct role in coculture interaction.

References

1. Kumagai, H., T. Fujiwara, H. Matsubara, and K. Saeki. 1997. Membrane localization, topology, and mutual stabilization of the rnfABC gene products in *Rhodobacter capsulatus* and implications for a new family of energy-coupling NADH oxidoreductases. *Biochemistry* 36 (18), 5509-5521
2. Pires, R.H., A.I. Lourenco, F. Morais, M. Teixeira, A.V. Xavier, L.M. Saraiva, I.A.C. Pereira. 2003. A novel membrane-bound respiratory complex from *Desulfovibrio desulfuricans* ATCC 27774, *Biochim. Biophys. Acta.* 1605: 67-82.
3. Zane, G.M., H. Yen, and J.D. Wall. 2010. Effect of the deletion of qmoABC and the promoter distal gene encoding a hypothetical protein on sulfate-reduction in *Desulfovibrio vulgaris* Hildenborough. *Appl. Environ. Microbiol.* 2010 0: AEM.00691-10
4. Venceslau, S.S., R.R. Lino and I.A.C. Pereira. The Qrc membrane complex, related to the alternative complex III, is a menaquinone reductase involved in sulfate respiration. *J. Biol. Chem.* jbc.M110.124305. First Published on May 24, 2010.

Publication list (including patents) acknowledging the DOE grant or contract

1. Li, X., Q. Luo, N.Q. Wofford, K.L. Keller, M.J. McInerney, J.D. Wall and L.R. Krumholz. 2009. A molybdopterine oxidoreductase is involved in H₂ oxidation in *Desulfovibrio desulfuricans* G20. *J. Bacteriol.* 191: 2675–2682.
2. Zane, G.M., Yen, H.B. and J.W. Wall. Effect of the Deletion of qmoABC and the Promoter-Distal Gene Encoding a Hypothetical Protein on Sulfate Reduction in *Desulfovibrio vulgaris* Hildenborough *Appl. Environ. Microbiol.*, 76:5500-5509.
3. Castañeda-Carrión, I.N., M. Whiteley and L.R. Krumholz. 2009. Characterization of pNC1, a small and mobilizable plasmid for use in genetic manipulation of *Desulfovibrio africanus*. *J. Microbiol. Methods.* 79:23-31.