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Recent Work

Title
Sulfate reducing capacity of a qmoabc deletion in desulfovibrio vulgaris hildenborough

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Abstract
The sulfite-reducing bacteria (SRB) are a heterologous group of anaerobic bacteria linked by their ability to respire the costly substrate sulfate as an electron acceptor and as a source of sulfur for cellular biosynthesis. All of the SRB organisms, of which D. vulgaris is a member, apparently share the same pathway for sulfate reduction, including an activation step involving the conversion of sulfate to adenosine-phosphosulfate (APS), which consumes two ATP equivalents. The enzyme complex involved in the activation step is APS-reductase, comprised of the two proteins, ApsA and ApsB. In D. vulgaris the apsB gene is predicted to be the first two genes in a six gene operon. The three genes that immediately follow apsBA are qmoABC (quinone-inactivating membrane-bound oxidoreductase) that are conserved in all the genomes of SRB sequenced to date. We have deleted these three genes (and a hypothetical protein predicted to be present at the end of the operon, Dvu0751) in D. vulgaris and monitored the strain’s ability to grow in the presence of sulfate or sulfite. Here we describe the method of deleting these four genes and the growth characteristics of the construct. As predicted by its genomic location, the Qmo complex is essential for APS reduction and sulfate respiration but not sulfite respiration.

Background (Figures 1a, 1b)
Two basic means to reduce sulfate: assimilative (used for amino acid synthesis in non-SRB) and dissimilative (used for sulfate respiration in SRB).

D. vulgaris contains the enzymes for both types of sulfate utilization (fig. 1a). The operon containing the genes for dissimilative sulfate reduction, adenylylsulfate reductase, apsBA, also contains the genes qmoABC (an electron transport carrier) and a hypothetical protein (fig. 1b).

Construction of Δqmo strain (Figure 2)
Cloning without restriction enzyme scheme

Construction of qmo+ strain (Figure 3)

Verification of Δqmo (Figures 3a, 3b)
- Putative Δqmo mutants were selected by resistance to G418 and sensitivity to spectinomycin
- Southern blot verified double-homologous recombination (fig. 3a)
- Verified expression of apsA gene with a Northern blot (fig. 3b).

Verification of qmo+ (Figures 6a, 6b)
- Putative qmo+ colonies were screened by resistance to spectinomycin and ability to grow on lactate-sulfate medium (fig. 6a).
- Further verification was performed by probing for the apsA gene in wild-type, Δqmo, and putative qmo+ (fig. 6b).

Growth Characteristics of Δqmo (Figure 4)
- Growth of Δqmo is not possible on lactate-sulfate (fig. 4).
- Growth of Δqmo on lactate-sulfite is reduced (fig. 4) but remains similar to wild-type for lactate-thiosulfate (fig. 4).

Growth Characteristics of qmo+ (Figure 7)
Wild-type D. vulgaris and qmo+ were grown on lactate-sulfate, lactate-sulfite, and lactate-thiosulfate media to compare growth (fig. 7).
- Growth of the qmo+ is comparable to that of wild-type on lactate-sulfate and lactate-sulfite (fig. 7).

Conclusions
- The QmoABC complex is necessary for sulfate reduction in D. vulgaris.
- No other transmembrane complex is able to replace the function of the QmoABC complex to deliver electrons to the ApsBA complex.
- The apsBA genes are differentially expressed depending on the presence or absence of sulfate.
- Complementation of qmoABC and hypothetical protein back into the Δqmo strain restores nearly wild-type sulfate reduction capability.

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