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Quantification of *Desulfovibrio vulgaris* Dissimilatory Sulfite Reductase Gene Expression during Electron Donor- and Electron Acceptor-Limited Growth[▽]

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Previous studies have suggested that levels of transcripts for *dsrA*, a gene encoding a subunit of the dissimilatory sulfite reductase, are not directly related to the rates of sulfate reduction in sediments under all conditions. This phenomenon was further investigated with chemostat-grown *Desulfovibrio vulgaris*. Under sulfate-limiting conditions, *dsrA* transcript levels increased as the bulk rates of sulfate reduction in the chemostat increased, but transcript levels were similar at all sulfate reduction rates under electron donor-limiting conditions. When both electron donor- and electron acceptor-limiting conditions were considered, there was a direct correspondence between *dsrA* transcript levels and the rates of sulfate reduction per cell. These results suggest that *dsrA* transcript levels may provide important information on the metabolic state of sulfate reducers.

Quantifying levels of transcripts for key genes in anaerobic sedimentary environments can provide insights into the metabolic state of microorganisms carrying out important processes in those environments (4, 5, 16, 17, 20, 24). Information can be gained about the relative rates of microbial metabolism under different conditions or the factors limiting the rates of growth and metabolism. Such information is especially useful in the design of bioremediation strategies because it indicates the level to which the genes for the desired bioremediation enzymes are being expressed and can suggest modifications in amendments to overcome nutrient limitations or other environmental stresses (24).

Methods to evaluate the metabolic state of sulfate-reducing bacteria are of interest because of the important role that sulfate reducers play in the biogeochemistry of aquatic sediments (32) and corrosion (8, 14), as well as in the degradation of organic contaminants (9). For example, some sulfate reducers can degrade aromatic hydrocarbons (13), including prevalent contaminants such as benzene (23) and polycyclic aromatic hydrocarbons (2, 3, 6, 12, 25), and stimulating the activity of aromatic hydrocarbon-degrading sulfate reducers can be an effective bioremediation strategy (1, 29).

Dissimilatory sulfite reductase catalyzes the final step in sulfate reduction, and sequences for this enzyme are highly conserved among sulfate reducers (18, 19, 31). Pure culture studies provided a preliminary indication that transcript levels of dissimilatory sulfite reductase genes might be related to rates of sulfate reduction (15, 26). However, relating transcript levels to rates of sulfate reduction in marine sediments was more problematic (5). Transcripts for *dsrA*, which encodes the alpha subunit of dissimilatory sulfite reductase, were quantified. There was a general trend that sediments with no sulfate reduction had low *dsrA* transcript levels and that *dsrA* levels were higher with active sulfate reduction. In sediments with similar sulfate concentrations, there was a direct correlation between the rates of sulfate reduction and *dsrA* transcript levels. However, in laboratory sediment incubations in which sulfate was continually being depleted over time, *dsrA* transcripts increased over time even though sulfate reduction rates did not increase (5). These results suggested that *dsrA* levels can only be related to sulfate reduction rates in sediments under very similar conditions, limiting the predictive value of *dsrA* transcript measurements over a range of environmental conditions.

In order to better understand the factors controlling *dsrA* transcript levels in sulfate reducers, *Desulfovibrio vulgaris* Hildenborough (ATCC 29579) was grown under strict anaerobic conditions (N_2:CO_2, 80:20 [vol/vol]) at 30°C in a freshwater medium (2.5 g liter^{-1} $NaHCO_3$, 0.25 g liter^{-1} NH_4Cl, 0.6 g liter^{-1} or 0.06 g liter^{-1} [for batch or continuous cultures, respectively] $NaH_2PO_4 \cdot H_2O$, 0.1 g liter^{-1} KCl, and trace mineral and vitamin solutions) (21) supplemented with a selenite-tungstate solution (400 mg liter^{-1} NaOH, 6 mg liter^{-1} $Na_2SO_3 \cdot 5H_2O$, 8 mg liter^{-1} $Na_2WO_4 \cdot 2H_2O$) and reduced with sodium sulfide (1 mM final concentration). Continuous culture conditions under electron donor (10 mM lactate, 10 mM sulfate) and electron acceptor (10 mM lactate, 2.5 mM sulfate) limitations were described previously (10). The methods for measuring substrates and products were as follows: for sulfate, ion chromatography (22); for fatty acids, high-pressure liquid chromatography (10); for sulfide, colorimetry (7); for protein, a bicinchoninic acid method (30); and for cell numbers, epifluorescence microscopy with acridine orange staining (27).

Total RNA was isolated with the Qiagen RNeasy mini kit (Qiagen, Inc., Valencia, CA) after digestion of the cell pellet with 50 mg ml^{-1} lysozyme. Primers DSR1F (forward 5′-AAG GAA CCC CGC ACC AAC-3′) and DSR1R (reverse 5′-TTA TCT CAG GTG TCT CTT GCG GT-3′) (position 1 to 102,

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TABLE 1. Data at steady-state conditions during continuous growth of *Desulfovibrio vulgaris*

Growth type and rate[a]	Acetate (mM)	Sulfate (mM)	Lactate (mM)	Biomass[b]
Electron donor (lactate) limited				
0.03	6.8 ± 0.2	7.6 ± 0.2	0 ± 0	48.5
0.04	7.4 ± 0.1	7.9 ± 0.4	0 ± 0	56.7
0.05	6.9 ± 0.1	7.4 ± 0.03	0 ± 0	56.2
0.06	7.5 ± 0.5	7.7 ± 0.5	0 ± 0	59.3
Electron acceptor (sulfate) limited				
0.03	5.3 ± 0.2	0.4 ± 0.2	1.8 ± 0.9	50.1
0.04	6.4 ± 0.3	0 ± 0	1.3 ± 0.5	54.5
0.05	5 ± 0.4	0.4 ± 0.3	2.5 ± 0.7	58.0
0.06	6.5 ± 0.4	0 ± 0	2.5 ± 0.2	43.7

[a] Specific growth rate (h^{-1}) or dilution rate in the continuous culture. Steady-state concentrations of acetate, sulfate, and lactate (average of the results from triplicate continuous cultures at a certain growth rate × 3 sampling events and standard deviation). Initial conditions: electron donor-limited growth, 10 mM lactate and 10 mM sulfate; electron acceptor-limited growth, 10 mM lactate and 2.5 mM sulfate.

[b] Biomass values are given in milligrams of dry weight per liter.

dsrA gene) were evaluated and optimized for quantitative PCR as previously described (4). Reverse transcription was performed with an Enhanced Avian First Strand synthesis kit (Sigma-Aldrich, St. Louis, MO). A dilution series of purified PCR products (10^5 to 10^{11} molecules) was the calibration standard for the real-time PCR quantification as previously described (16). The cDNA obtained from the reverse transcription reaction was quantified in a GeneAmp 5700 sequence detection system with a reaction mixture of primers (150 nM each), 9.5 μl of cDNA (20 to 250 ng μl^{-1}), 12.5 μl of Power Sybr green PCR master mix (Applied Biosystems, Foster City, CA) to a final volume of 25 μl. *dsr* transcript levels were normalized to the total RNA levels to help account for different biomass-specific sulfate reduction rates (28).

D. vulgaris was initially grown in chemostats in which electron-donor availability limited growth (Table 1). This was designed to simulate the conditions in superficial marine sediments in which sulfate is abundant. Under such conditions, the growth of sulfate reducers is primarily controlled by the rates at which complex organic matter is broken down into simpler substrates that sulfate reducers can utilize. In this system, bulk sulfate reduction rates (moles of sulfate reduced per liter per h) at steady state are analogous to the bulk sulfate reduction rates measured in sediments. The levels of *dsrA* transcripts were comparable over the range of sulfate reduction rates evaluated (Fig. 1A), suggesting that, as previously found in sediment studies (5), *dsrA* transcript levels are not always correlated with bulk rates of sulfate reduction. In order to investigate this further, a second set of chemostats (Table 1) were run in which the electron acceptor, sulfate, was the limiting nutrient (Fig. 1B). In contrast to the results from the electron donor-limited chemostats, in the electron acceptor-limited chemostats there was an increase in *dsrA* transcript levels that paralleled increasing sulfate reduction rates at increasing dilution rates (Fig. 1B).

Differences in the results of the electron donor- versus electron acceptor-limited chemostats could be reconciled when the levels of *dsrA* transcripts were compared with the rate of sulfate reduction per cell (Fig. 2). There was a strong correlation between this cell-specific sulfate reduction rate and *dsrA* transcript levels.

High levels of *dsrA* transcripts in the sulfate-limited chemostats could not be attributed solely to low sulfate levels. For example, when *D. vulgaris* grown in sulfate-free medium either with pyruvate as a fermentative substrate or with lactate as the electron donor in coculture with *Methanospirillum hungatei* (DSM864), *dsrA* levels were low ($1.06 \cdot 10^4 \pm 2{,}000$ and $8.6 \cdot 10^4 \pm 8{,}000$ transcripts per nanogram of RNA, respectively).

A comparison of specific sulfate reduction rates and bulk sulfate reduction rates in the electron donor-limited and electron acceptor-limited chemostats demonstrated that electron acceptor-limited cells consistently have a higher respiration rate per cell (Fig. 3). This is consistent with an apparently higher maintenance energy requirement (calculated as previously described by Esteve-Nuñez et al. [10]) under electron acceptor-limited conditions (6.3 mmol electrons per gram dry weight per h) than under electron donor-limited conditions (2.7 mmol electrons per gram per h). Higher expression of

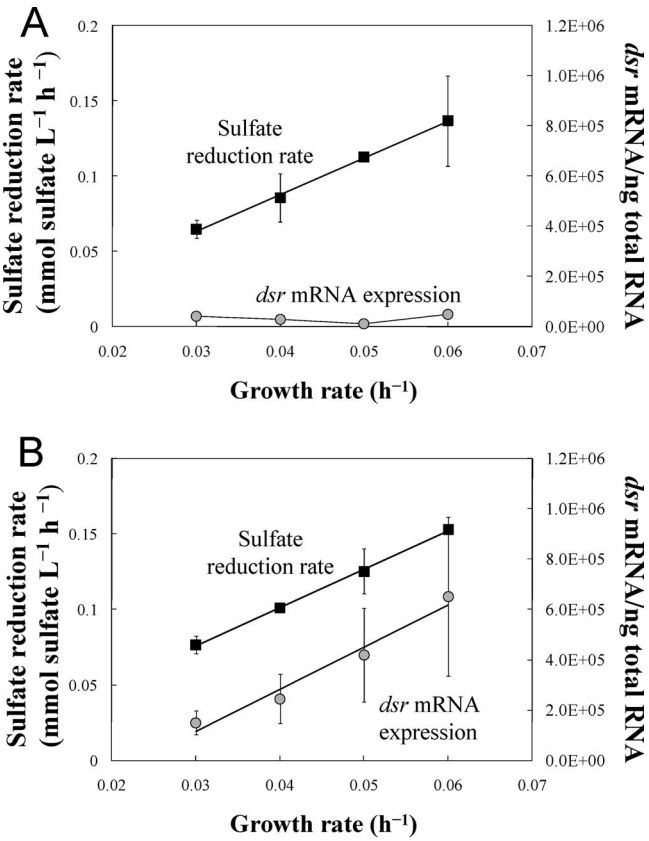

FIG. 1. Sulfate reduction rate (mmol sulfate consumed at steady state per liter and h; black squares) and number of *dsrA* mRNA transcripts (gray circles) expressed by *D. vulgaris* cells grown under electron donor (A) or electron acceptor (B) limitation at different growth rates. Each point is the average of the results for three samples from triplicate continuous cultures at each growth rate. (A) Sulfate reduction rate, $R^2 = 0.997$. (B) Sulfate reduction rate, $R^2 = 0.998$; *dsrA* mRNA expression, $R^2 = 0.967$.

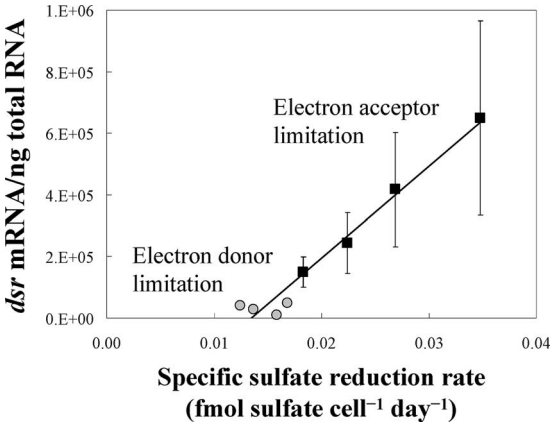

FIG. 2. *dsr* mRNA transcripts expressed by *D. vulgaris* cells grown under electron donor (gray circles) and acceptor (black squares) limitation at a specific sulfate reduction rate (femtomol sulfate consumed per cell and day). Each point is the average of the results for three samples from triplicate continuous cultures at each growth rate. R^2 = 0.964.

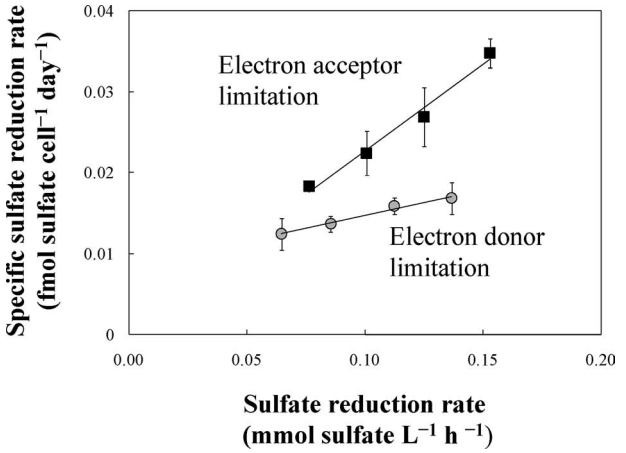

FIG. 3. Relationship between the specific sulfate reduction rate (femtomol sulfate consumed per cell and day) and the bulk sulfate reduction rate (mmol sulfate consumed per liter and h) in *D. vulgaris* continuous culture under electron donor (gray circles; R^2 = 0.9894) and acceptor (black squares; R^2 = 0.983) limitation. Each point is the average of the results for three samples from triplicate continuous cultures at each growth rate.

respiratory genes under electron acceptor-limiting versus electron donor-limiting conditions was previously noted in chemostat cultures of *Geobacter sulfurreducens* grown with either fumarate or Fe(III) as the electron acceptor (4).

Implications. These results suggest that the level of *dsrA* transcripts in an environmental sample cannot be expected to be related to the bulk rates of sulfate reduction because the relationship between *dsrA* transcript levels and the rates of sulfate reduction can be influenced by the metabolic state of the cell. This may explain why in a previous study (5) there was a good correlation between *dsrA* transcript levels and sulfate reduction rates at comparable nonlimiting sulfate concentrations, but *dsrA* levels continued to increase as sulfate concentrations declined even though sulfate reduction rates decreased. The inability of *dsrA* transcript levels to serve as a proxy for bulk sulfate reduction rates may not be a significant limitation for the study of sulfate reduction in most environments because several other more straightforward methods, such as monitoring the reduction of [^{35}S]sulfate to [^{35}S]sulfide (11), are available for estimating bulk sulfate reduction rates.

However, the results from this pure culture study suggest that *dsrA* transcript levels can provide insight into the metabolic state of sulfate reducers because *dsrA* levels are related to the rates of sulfate reduction per cell. This basic information on the metabolic state of sulfate reducers cannot readily be determined with any other method currently applicable to sedimentary environments. For studies with natural communities, the normalization of *dsrA* transcript levels to total RNA levels, as used here for pure cultures, might not be appropriate because many organisms will contribute to the RNA pool. One solution that has proven successful in diagnosing the metabolic status of subsurface Fe(III)-reducing communities is to normalize to the transcript levels of a housekeeping gene sequence specific for the organisms under study (16, 17). Thus, further studies are warranted to determine whether the relationship between *dsrA* expression and cell-specific rates of sulfate reduction observed here in pure culture holds for diverse communities typically found in sedimentary environments.

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