Molecular analysis of the metabolic rates of discrete subsurface populations of sulfate reducers

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Short title: Molecular analysis of metabolic rates of sulfate reducers

Abstract

Elucidating the *in situ* metabolic activity of phylogenetically diverse populations of sulfate-reducing microorganisms that populate anoxic sedimentary environments is key to understanding subsurface ecology. Previous pure culture studies have demonstrated that transcript abundance of dissimilatory (bi)sulfite reductase genes is correlated with the sulfate-reducing activity of individual cells. To evaluate whether expression of these genes was diagnostic for subsurface communities, dissimilatory (bi)sulfite reductase gene transcript abundance in phylogenetically distinct sulfate-reducing populations was quantified during a field experiment in which acetate was added to uranium-contaminated groundwater. Analysis of *dsrAB* sequences prior to the addition of acetate indicated that *Desulfobacteraceae*, *Desulfobulbaceae*, and *Syntrophaceae*-related sulfate reducers were the most abundant. Quantifying *dsrB* transcripts of the individual populations suggested that *Desulfobacteraceae* initially had higher *dsrB* transcripts per cell than *Desulfobulbaceae* or *Syntrophaceae* populations, and that the activity of *Desulfobacteraceae* increased further when the metabolism of dissimilatory metal reducers competing for the added acetate declined. In contrast, *dsrB* transcript abundance in *Desulfobulbaceae* and *Syntrophaceae* remained relatively constant, suggesting a lack of stimulation by added acetate. The indication of higher sulfate-reducing activity in the *Desulfobacteraceae* was consistent with the finding that *Desulfobacteraceae* became the predominant component of the sulfate-reducing community. Discontinuing acetate additions resulted in a decline in *dsrB* transcript abundance in the *Desulfobacteraceae*. These results suggest that monitoring transcripts of dissimilatory (bi)sulfite reductase genes in distinct populations of sulfate reducers can provide insight into the relative rates of metabolism of

different components of the sulfate-reducing community and their ability to respond to environmental perturbations.

Keywords: dissimilatory (bi)sulfite reductase (DSR) / gene expression / Rifle IFRC / subsurface / U(VI) bioremediation / Sulfate-reducing prokaryotes (SRP)

Introduction

A major goal of microbial ecology is to know not only what microorganisms are present, and the metabolic potential of those organisms as revealed in their genomes, but also to understand key *in situ* physiological characteristics, such as rates of metabolism of individual components of the community. Dissimilatory sulfate reduction has a key role in the global sulfur cycle and represents one of the most important organic matter mineralization processes in a diversity of environments. Sulfate-reducing prokaryotes (SRP) can colonize a variety of niches in marine (11, 13, 26, 55), brackish (27, 32), freshwater (3, 7, 30, 33, 43, 52, 56), and extreme environments (22, 24, 37, 59). SRP are also of interest for their economical relevance in the remediation of naturally or anthropogenically-contaminated habitats (1, 9, 16, 18, 23, 25), and their involvement in the corrosion of metallic oil, gas or potable water pipelines (38, 48, 50).

Studies on chemostat cultures of *Desulfovibrio vulgaris* demonstrated that transcript abundance for the gene *dsrA*, which encodes the α subunit of the dissimilatory (bi)sulfite reductase (12) was directly proportional to the sulfate reduction rate in individual cells and that sulfate reduction rates per cell varied significantly depending upon growth rates of the cells and whether the growth of the cells was limited by the availability of electron acceptor or electron

donor (57). Thus, abundance of *dsrA* transcripts in sediments (10) cannot be used to estimate bulk rates of sulfate reduction without acquiring additional physiological data not readily obtained with current environmental technologies . However, *dsrA* transcript abundance can be a guide to the metabolic rate of the individual cells in that environment.

SRP are phylogenetically and physiologically diverse. Although unified by their sulfate-reducing ability, SRP are polyphyletic (i.e. they can be divided in four distinct bacterial phyla and one archaeal phylum), comprising more than 150 cultured species divided into 40 genera (17). Depending on the species, SRP couple the oxidation of H_2 or a variety of carbon substrates to acetate (incomplete oxidizers) or CO_2 (complete oxidizers), to the reduction of sulfate or alternative (in)organic (non-)sulfur electron acceptors (47). In the absence of electron acceptors, SRP are also able to perform fermentation (47). Therefore, in order to better understand the *in situ* physiology of sulfate-reducing microorganisms, it would be beneficial to separately track the metabolism of physiologically distinct populations of sulfate reducers.

One feature in which sulfate reducers differ significantly is their ability to reduce U(VI). Microbial U(VI) reduction is expected to play an important role in the natural cycling of uranium (28). Furthermore, it is an attractive bioremediation tool because reduction of highly soluble U(VI) to poorly soluble U(IV) can be an effective strategy for reducing the mobility of uranium in contaminated subsurface environments (14). Some sulfate reducers such as *Desulfovibrio* sp. (29), *Desulfotomaculum reducens* (54), and *Desulfosporosinus* sp. (53) are effective U(VI) reducers, whereas others such as *Desulfobacter postgatei*, *Desulfobulbus propionicus*, and *Desulfobacca acetoxidans* (29) are not. Therefore, information on which populations of sulfate reducers are active under different conditions could greatly aid in the design of strategies for

groundwater uranium bioremediation and better constrain the metabolic diversity underlying enzymatic removal processes during uranium bioremediation.

Here we demonstrate that it is possible to track the activity of different populations of sulfate reducers by individually monitoring transcript abundance for dissimilatory (bi)sulfite reductase genes for each population.

Material and Methods

Site description

During July to September 2008, a study on bioremediation of uranium-contaminated groundwater was conducted at the Department of Energy (DOE) Rifle Integrated Field Research Challenge (IFRC) site near Rifle, CO (USA) (2, 58, 61). Briefly, the site is a floodplain of the Colorado River located in Northwestern Colorado. The aquifer is a ~6.5 m thick heterogeneous alluvial deposit consisting of unconsolidated clay, silt, sand, gravels, and cobbles lying on weathered claystone of the Wasatch formation. The groundwater table is ~3.5 m below surface and the flow is towards the Colorado River. The experimental plot was a 12 m by 18 m flow cell comprised of 3 upgradient monitoring wells, 10 injection wells, and 12 downgradient monitoring wells (Fig. 1). Groundwater samples for chemical and molecular analyses were taken from the representative well D04. This is an anoxic site, as demonstrated by the presence of Fe(II) in the groundwater (34, 61), and nitrate is not available as an electron acceptor (34).

Groundwater amendment and sampling

As previously described (61) an acetate:bromide solution was prepared by mixing native groundwater pumped from an upgradient portion of the aquifer into a storage tank with sodium acetate (SIGMA, Saint Louis, MO, USA) and sodium bromide (SIGMA). This mixture was added to the subsurface via 10 injection wells to achieve target aquifer concentrations of ~5 mM and ~1 mM, for the first 14 days. Additions resumed on day 25 and on day 38 the acetate concentration was increased to provide a target concentration of ~15 mM with continued additions to day 110 (61). However, a diversion in groundwater flow and acetate consumption at the injection wells diminished the delivery of the injectate to D04 after the groundwater flush (61).

Prior to the initiation of the acetate injection reported here, the site had been under natural groundwater flow without amendments for ~11 months, following a previous, short-duration (ca. 21-day) acetate-addition study in 2007 (61).

Groundwater samples for geochemical analyses were collected every two days after purging 10 L of groundwater from the well using a peristaltic pump. Sulfide and ferrous iron were measured spectrophotometrically immediately after sampling using the methylene blue method (Hydrogen Sulfide Test, HACH Company, Loveland, CO, USA) for sulfide, and the phenanthroline method (AccuVac® Ampuls, HACH Company) for ferrous iron. After filtration through a 0.2 μm pore size PTFE (Teflon) filter (Alltech Associates Inc., Deerfield, IL, USA), bromide, acetate and sulfate concentrations were measured using an Dionex ICS-1000 ion chromatograph equipped with a IonPac® AS22 column, a ASRS® 300 suppressor, and 4.5 mM carbonate/1.4 mM bicarbonate eluent (Dionex Corporation, Sunnyvale, CA, USA), while U(VI) was measured using a kinetic phosphorescence analyzer (46).

Groundwater samples for molecular analyses were obtained after sampling for geochemical analyses by concentrating 10 L of groundwater on a 0.2 µm, 293 mm diameter Supor®-200 membrane filter (ø=293 mm, pore size = 0.2 µm; Pall Life Sciences, Ann Arbor, MI, USA). Filters were quickly sealed into a sterile whirl-pack, flash frozen in an ethanol-dry ice bath, and stored at –80°C until nucleic acid extraction.

Nucleic acid extraction

Nucleic acids were extracted from portions of the same filter and crushed with liquid nitrogen (34). Equal volumes of homogenized filter fragments were used for parallel DNA and RNA extractions. Genomic DNA (gDNA) was extracted using the FastDNA® SPIN Kit for soil (MP Biomedicals, Solon, OH, USA). gDNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and stored at –80°C until further analyses.

RNA was extracted using a modified phenol-chloroform method (20). RNA clean up was performed using the RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany) and treated with the DNase (DNA-freeTM Kit, Ambion, Austin, TX, USA). Successful RNA isolation was checked by visualization on a 1% agarose gel. The absence of DNA contamination was confirmed by PCR amplification. RNA was quantified using a NanoDrop spectrophotometer and stored at –80°C until further analyses.

dsrAB *clone libraries construction and phylogenetic analysis*

The primers used in this study are listed in Table 1. PCR amplification of an approximately 1.9-kbp *dsrAB* fragment was performed using the primers DSR1Fmix (equimolar

mixture of DSR1F, DSR1Fa, DSR1Fb, DSR1Fc, and DSR1Fd) and DSR4Rmix (equimolar mixture of DSR4R, DSR4Ra, DSR4Rb, DSR4Rc, DSR4Rd, and DSR4Re), and the following cycling conditions: initial denaturation at 94˚C for 1 min, 35 cycles of 40 s denaturation at 94˚C, 40 s annealing at 48˚C, 1.5 min elongation at 72˚C, and final elongation at 72˚C for 10 min (30). A positive control of purified *dsrAB* PCR product from *Desulfovibrio vulgaris*, and a negative control without DNA were always included in PCR amplification experiments. The reaction was carried in a PTC200 Peltier Thermal Cycler (MJ Research, Waltham, MA, USA). The 50 µL reaction mixture contained 100 ng of DNA, 1X Q-Solution (QIAGEN), 1X PCR Buffer (QIAGEN), 1.5 mM $MgCl_2$ (QIAGEN), 200 µM concentrations of each deoxynucleotide (SIGMA), 0.5 µM concentrations of each primer, 0.5X BSA (New England Biolabs, Beverly, MA, USA), and 1.25 U of Taq DNA polymerase (QIAGEN). The presence and size of the amplification products were determined by agarose (1% [w/v]) gel electrophoresis. Bands of the expected size were purified from the gel by excision with a sterile surgical blade and purified with the QIAquick Gel Extraction Kit as recommended by the manufacturer (QIAGEN).

Clone libraries were constructed from 9 representative samples (day 0, 3, 10, 13, 26, 34, 45, 47, and 53 following acetate injection). Four µL of the agarose gel-purified DNA mixture was ligated into the pCR2.1®-TOPO® vector (TOPO TA Cloning® Kit, Invitrogen, Carlsbad, CA, USA). A *dsrAB* fragment sequence of approximately 1.9-kbp was determined for *E. coli* recombinant vector-containing colonies with the primers M13F and M13R, in an ABI 3730xl DNA Analyzer using the Sanger chain-terminator method with fluorescently labeled nucleotides. Chromatograms were visually inspected using the software 4Peaks v1.7 (Griekspoor and Groothuis, [http://www.mekentosj.com]).

Recovered *dsrAB* sequences (100 clones per library) were compared to GenBank database (4) for preliminary identification using the BLASTX algorithms ([http://www.ncbi.nml.nih.gov/BLAST]). The alignment and treeing software of the ARB package (31) [http://www.arb-home.de]) were used for the phylogenetic analyses. Concatenated partial *dsrA* and *dsrB* sequences were added to an ARB alignment of 1.9-kb *dsrAB* sequences (63) deposited in the GenBank database. The alignment of the corresponding amino acid sequences was carried out manually using the editor GDE 2.2 (51) implemented in ARB. A *dsrAB* tree was constructed from nucleotide sequences using neighbor-joining analysis with Jukes-Cantor distance correction. The trees constructed with nucleotide and amino acids sequences yielded similar results. Phylogenetic inference was performed with a total of 1123 nucleotides; filters were used to exclude from the dataset regions of insertion and deletions, as well as the third position in each triplet. Representative concatenated partial *dsrA* and *dsrB* nucleotide sequences determined in this study have been submitted to the NCBI database under accession numbers HQ690090-HQ690096.

Primer design for quantifying dsrB *transcripts*

Conserved regions in the alignment of sequence data from the *dsrAB* clone libraries were targeted for quantitative PCR (qPCR) primer design. The primer pairs DSRq1F-DSRq1R, DSRq2F-DSRq1R, and DSRq4F-DSRq1R (Table 1) were employed to amplify a portion of 105, 110, and 115 bp of the *dsrB* portion of the *dsrAB* of sulfate reducers belonging to the *Desulfobacteraceae*, *Desulfobulbaceae* and *Syntrophaceae* clusters found in the groundwater at Rifle, respectively. The specificity of the primer pairs was tested *in silico* using the ARB software. In addition, clone libraries were constructed from PCR-amplified DNA fragments from

DNA extracted from the sampling filters using each primer pair and the protocol described above. Proper matching with the targeted SRP was confirmed by inserting the partial *dsrB* sequences one by one into the tree constructed with long *dsrAB* sequences using the ARB parsimony tool, without distorting the overall tree topology (data not shown).

RT-PCR of dsrB *transcripts*

The Enhanced Avian HS RT-PCR Kit (SIGMA) was used to generate complementary DNA (cDNA) from extracted *dsrAB* transcripts. The reverse transcription (RT) reaction was carried on in two steps. First, the RT master mix contained 2 µL of the appropriate reverse primer (2 µM), 2 µL of deoxynucleotide mix (1 mM each dNTP), 1 µL of DEPC-treated water; 5 µL of RNA template (0.01-5 µg RNA) were added for a total reaction volume of 10 µL, and the mixture was incubated at 70°C for 10 min. Then, the PCR master mix (10 µL) consisted of 2 µL of AMV Reverse Transcriptase Buffer (1X), 1 µL RNase inhibitor (1 U/µL), 1 µL Enhanced AMV Reverse Transcriptase (1 U/µL), and 6 µL DEPC-treated water, was added to the RT reaction mixture and the samples were incubated at 50°C for 50 min. cDNA was quantified using a NanoDrop spectrophotometer and stored at –80°C until further analyses.

Quantification of genes and transcripts

The 25 µL qPCR reaction mixture contained 12.5 µL of *Power* SYBR Green PCR Master Mix (Applied Biosystems Inc., Foster City, CA, USA), 1.5 µL of 150 nM concentrations of each primer, and 9.5 µL of a 1:10 dilution of gDNA (*dsrB*) or cDNA (*dsrB* transcripts) template. qPCR results were normalized to the total amount of gDNA/cDNA in the 9.5 µL of template solution used to set up the qPCR reaction. Standard curves were constructed with serial dilutions

of known amounts of *dsrB* amplified with the appropriate primers from environmental gDNA, purified and quantified with a NanoDrop spectrophotometer. Serial dilutions covered a range of 8 orders of magnitude of template copies per assay (10^2-10^9). R^2 values ranged from 0.992-0.999. The qPCR efficiency (90%-95%) was calculated based on the slope of the standard curve. All qPCR assays were run in triplicate. PCR amplification was carried on with the 7500 Real-Time PCR System (Applied Biosystems). Thermal cycling parameters consisted of an activation step at 50°C for 2 min, a denaturation step at 95°C for 10 min, and 50 cycles at 95°C for 15 s and 55°C for 1 min. Amplification and correct amplicon size were verified running aliquots of qPCR reactions on an ethidium bromide-stained 1% agarose gel. gDNA extracts were tested for PCR inhibitory substances by a serial dilution of the template gDNA and subsequent qPCR. Templates were normalized to an equal amount of gDNA/cDNA to enable comparison of different time points.

Results and Discussion

Evidence for acetate additions driving sulfate reduction

As previously reported (61) acetate concentrations in groundwater pumped from well D04 initially increased in response to injection (Fig. 2). As soon as acetate was introduced there appeared to be an increase in sulfate reduction, as evidenced by a decline in sulfate over time, as well as a stimulation of dissimilatory metal reduction, as evidenced by a decline in U(VI) (Fig. 2). It is assumed that there was also a stimulation of dissimilatory Fe(III) reduction during this same period, but this is difficult to ascertain from groundwater geochemistry. Concentrations of dissolved Fe(II) are not a good proxy for Fe(III) reduction in the subsurface, as most of the Fe(II)

produced from dissimilatory metal reduction typically remains in solid phases and Fe(II) concentrations in the groundwater merely reflect geochemical equilibria with multiple Fe(II) phases (39). The simultaneous initiation of sulfate reduction and dissimilatory metal reduction with the addition of acetate can be attributed to the fact that acetate had been added to this site in the previous year and had already begun to enrich for sulfate reducers that could compete with *Geobacter* species for acetate (5). With continued acetate addition, dissolved sulfide began to accumulate in the groundwater, providing additional evidence for sulfate reduction.

Between days 15 and 24 no additions were made to the groundwater, and at day 25 when acetate injections were resumed acetate concentrations were undetectable (<0.1 mM) and sulfate and uranium concentrations had rebounded (Fig. 2). As previously reported (61), delivery of the injectate to D04 was diminished from day 25 as indicated by low levels of the bromide tracer reaching this location (Fig. 2). However, sulfate reduction rates appeared to accelerate as evidenced by a more rapid depletion of sulfate over time than what was observed in the initial phase of acetate additions. The undetectable (<0.01 mM) levels of bromide by day 50, coupled with a rebound in sulfate concentrations, suggested that acetate was no longer being delivered to D04 by this time. U(VI) concentrations remained high following the resumption of acetate additions, which is consistent previous studies that have noted a lack of U(VI) removal during active sulfate reduction (2, 58, 61).

Sulfate reducers present

To make a comprehensive inventory of the SRP present at the Rifle site, *dsrAB* clone libraries were constructed from DNA extracted from samples representative of the entire experimental period. Seven groups of *dsrAB* sequences were retrieved, with 99-100% sequence

identity within each group. One group was in the *Desulfobacteraceae*, three were in the *Desulfobulbaceae*, and three were in the *Syntrophaceae* (Fig. 3). Analysis of *dsrAB* sequences revealed that three clades of sulfate reducers at the site: *Desulfobacteraceae* (*dsrAB* clone Rifle08_01), *Desulfobulbaceae* (*dsrAB* clones Rifle08_02-04) and *Syntrophaceae* (*dsrAB* clones Rifle08_05-07; Fig. 3). The closest cultured relatives to the *Desulfobacteraceae* and *Syntrophaceae* sequences are the acetate-oxidizing sulfate reducers *Desulfobacter postgatei* (96-97% sequence identity) and *Desulfobacca acetoxidans* (69-72% sequence identity), respectively. The *Desulfobulbaceae* sequences were not closely related to known acetate-oxidizing sulfate reducers. Primer DSR1F and DSR4R mixes used in this study were recently implemented with additional variants to improve *dsrAB* coverage (45). Hence, we do not exclude the possibility that our survey underestimated the *dsrAB* diversity in the groundwater at Rifle.

Prior to the addition of acetate, *Desulfobulbaceae* and *Desulfobacteraceae* were comparable in abundance (Fig. 4). However, following the addition of acetate *Desulfobacteraceae* became predominant and the proportion of *Desulfobulbaceae* declined significantly. *Syntrophaceae* had lower abundance prior to acetate additions, and remained present at a comparable abundance throughout. This specific response of *Desulfobacter* to the acetate additions was corroborated with 16S rRNA sequence analysis performed with microarrays (8).

Expression of dissimilatory (bi)sulfite reductase gene in the three clades

The activity of the three major clades of sulfate reducers throughout the field study was evaluated by monitoring the abundance of *dsrB* transcripts in each group. The number of *dsrB* transcripts in each clade was normalized to the number of copies of *dsrB* in that clade. SRP that

possess multiple *dsr* operons in their genome have not been reported, and thus this normalization is expected to approximate *dsrB* transcripts per cell.

The *Desulfobacteraceae* had a slightly higher abundance of *dsrB* transcripts than the *Desulfobulbaceae* or *Syntrophaceae* prior to the addition of acetate to the subsurface (Fig. 5). Following the resumption of acetate additions on day 25, the abundance of transcripts in the *Desulfobacteraceae* increased. This coincided with the enhanced rate of sulfate removal noted above, consistent with higher activity of sulfate reducers. When acetate was no longer being delivered to D04 as indicated by diminished bromide and a rebound in sulfate concentrations, *dsrB* transcript abundance in *Desulfobacteraceae* declined rapidly, consistent with the expected decline in the activity of sulfate reducers.

In contrast, the abundance of *dsrB* transcripts remained relatively constant in the *Desulfobulbaceae* throughout the field experiment. The abundance of *dsrB* transcripts in the *Syntrophaceae* increased slightly in the later phases of sulfate reduction, but remained low compared to transcript abundance in the *Desulfobacteraceae* (Fig. 5).

These results suggest that, on a per cell basis, *Desulfobacteraceae* were much more responsive to the changes in the availability of acetate than the other two groups of sulfate reducers. This interpretation is consistent with the finding that *Desulfobacteraceae* became the most dominant group of sulfate reducers following acetate addition.

Implications

These results suggest that, with appropriate design of primers, it is possible to specifically monitor gene expression for the respiratory enzyme dissimilatory (bi)sulfite reductase in different clades of sulfate reducers. Previous studies have analyzed dissimilatory (bi)sulfite gene

sequences to describe the distribution of phylogenetically distinct populations of sulfate reducers in a diversity of environments (6, 22, 24, 26, 44, 49). As shown here, when transcript abundance of the dissimilatory (bi)sulfite reductases of different populations is quantified, it is possible to evaluate how the metabolism of competing populations of sulfate reducers shifts in response to changing environmental conditions.

If it is assumed that there is a direct relationship between *dsrB* transcript abundance and rates of sulfate reduction per cell, as has been described in pure culture studies (57), then the results suggest major differences in the ability of the different clades of sulfate reducers to respond to added acetate. Whereas members of the *Desulfobulbaceae* were competitive with *Desulfobacteraceae* at the Rifle site in the absence of added acetate, *Desulfobacteraceae* were able to increase their per cell rates of sulfate reduction more effectively and thus outcompete the *Desulfobulbaceae* once acetate was added. *Syntrophaceae* were also able to increase respiration rates in the presence of added acetate, but not to the levels of the *Desulfobacteraceae*.

The different responses of the individual clades may be related, at least in part, to which electron donors they are capable of utilizing. *Desulfobacter* species can effectively metabolize acetate (47), whereas no species of *Desulfobulbaceae* are known to use acetate (15, 47).

However, other physiological features may also play a role. The *Syntrophaceae* sequences retrieved were related to the known acetate-oxidizer *Desulfobacca acetoxidans* (41), suggesting that the *Syntrophaceae* most abundant at the Rifle site were also likely to be capable of acetate consumption. Multiple factors other than the ability to use acetate are likely to determine the outcome of competition for added acetate. For example, genome-scale modeling of the competition between acetate-oxidizing, Fe(III)-reducing *Geobacter* and *Rhodoferax* species at the Rifle site demonstrated that the predominance of these two species under different

conditions could be attributed to differences in growth yield, specific growth rates, and the capacity for nitrogen fixation (62).

The nutritional requirements for growth on acetate of the cultured *Desulfobacca acetoxidans* (41) is comparable to that of cultured *Desulfobacter* species (60), but the mean specific growth rate of *Desulfobacter* species (μ_{max}= 0.8-1.1 d^{-1}; 42) is approximately twice as fast as that of *Desulfobacca acetoxidans* (μ_{max}= 0.3-0.4 d^{-1}; 41). Higher growth rate is a key factor permitting *Geobacter* species to outcompete *Rhodoferax* species when acetate is added at the Rifle site (35, 62). Furthermore, the *Syntrophaceae*-related *dsrAB* gene sequences recovered at the Rifle site are only moderately similar to the *Desulfobacca acetoxidans* sequence, and thus it is conceivable that the *Syntrophaceae*-related organisms at Rifle might not have the same ability to metabolize acetate.

Analysis of expression of key genes indicative of the physiological status of *Geobacter* species within the subsurface community at the Rifle site has provided further insight into the factors controlling the growth of these organisms following acetate addition (19-21, 34-36, 40, 42, 60). A similar in-depth transcriptional profiling of the sulfate-reducing community is warranted. The approach described here for elucidating the relative activity of different components of the sulfate-reducing community should be applicable to a diversity of environments.

Acknowledgements

We thank the city of Rifle, CO, the Colorado Department of Public Health and Environment, and the U.S. Environmental Protection Agency, Region 8, for their cooperation in this study. The

U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research funded the work under grant no. DE-SC0004814 (University of Massachusetts) and contract no. DE-AC02-05CH11231 (Lawrence Berkeley National Laboratory [LBNL; operated by the University of California], with support derived equally from the Rifle IFRC and LBNL Sustainable System Science Focus Area research programs).

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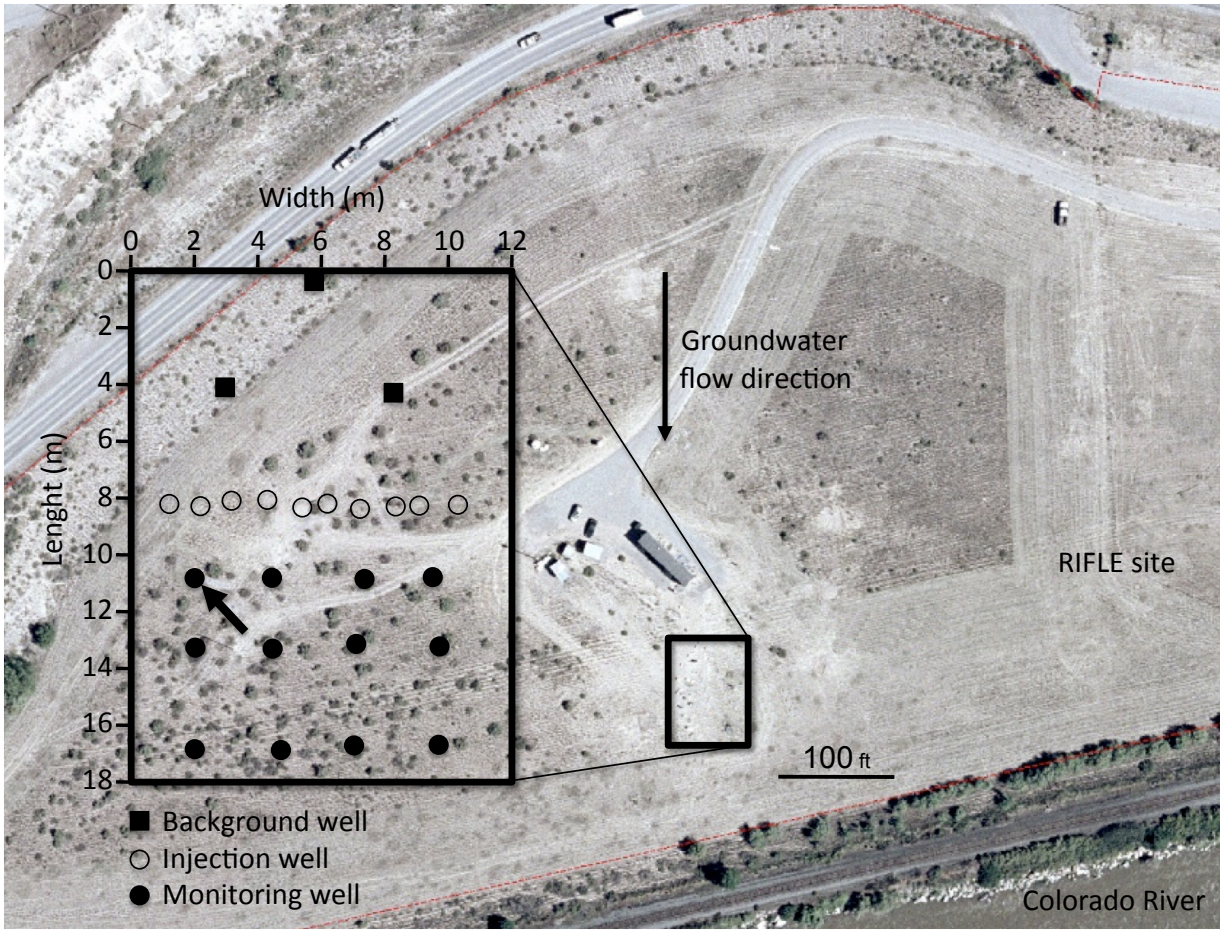

Figure 1. Aerial view of the uranium bioremediation field site in Rifle, CO. Within the flow cell, the arrow indicates the well sampled for chemical and molecular analyses (well D04).

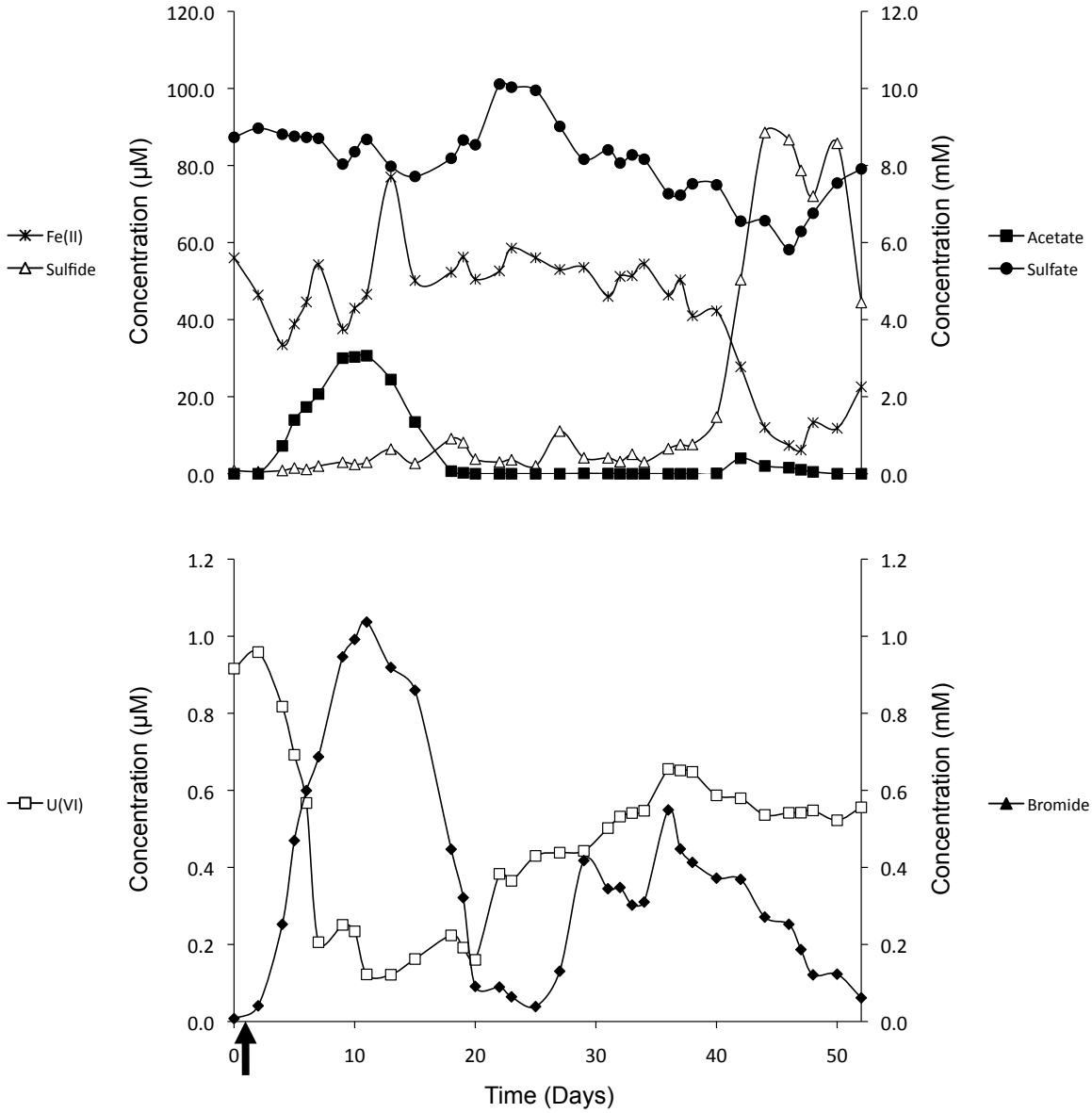

Figure 2. Fe(II), sulfide, acetate, sulfate, U(VI), and bromide concentrations in well D04 during acetate amendment at the Rifle site. The left y-axis refers to Fe(II), sulfide (upper panel), and U(VI) (lower panel) concentration. The right y-axis refers to acetate, sulfate (upper panel) and bromide (lower panel) concentration. Black arrows on the x-axis indicate the beginning of acetate injection in the subsurface.

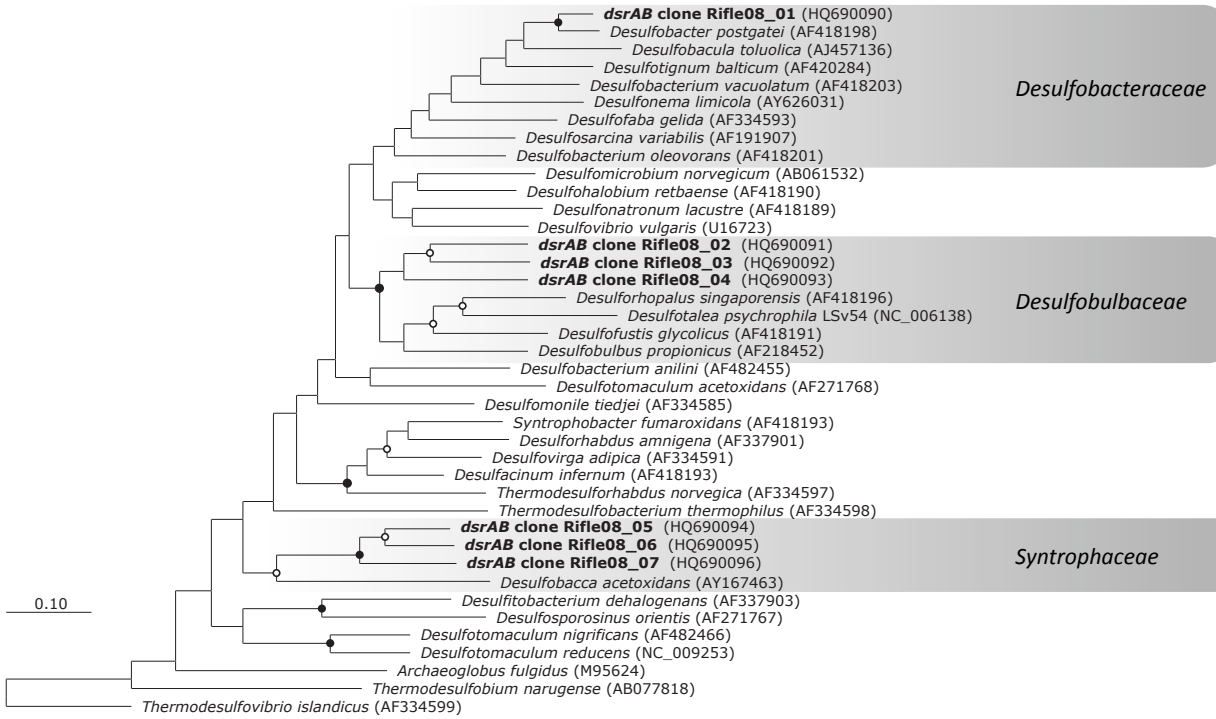

Figure 3. Phylogenetic tree showing the placement of a representative of each group of the *dsrAB* sequences recovered from the subsurface (in bold) as well as sequences from pure cultures. The tree was constructed using the neighbor-joining algorithm using full *dsrAB* sequences for cultured SRP (63), and concatenated *dsrA* and *dsrB* for clones. Closed circles indicate bootstrap values (1000 data resamplings) of ≥90%, open circles indicate values of ≥70%. The *dsrAB* sequence of *Thermodesulfovibrio islandicus* was used as outgroup. The scale bar indicates 10% sequence divergence. GenBank accession numbers are indicated for each sequence.

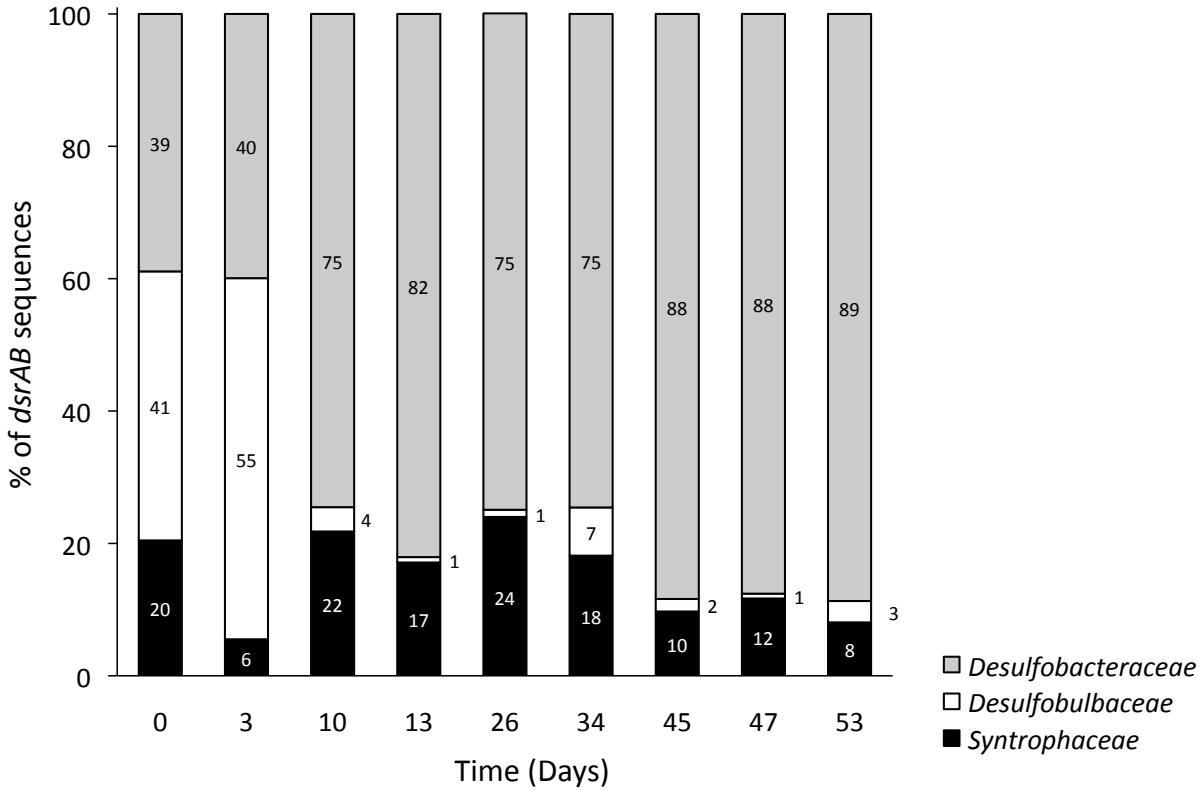

Figure 4. Relative abundance of the three sulfate-reducing clades in *dsrAB* clone libraries. Numbers indicate the number of clones belonging to each group from a total of 100 clones analyzed per library.

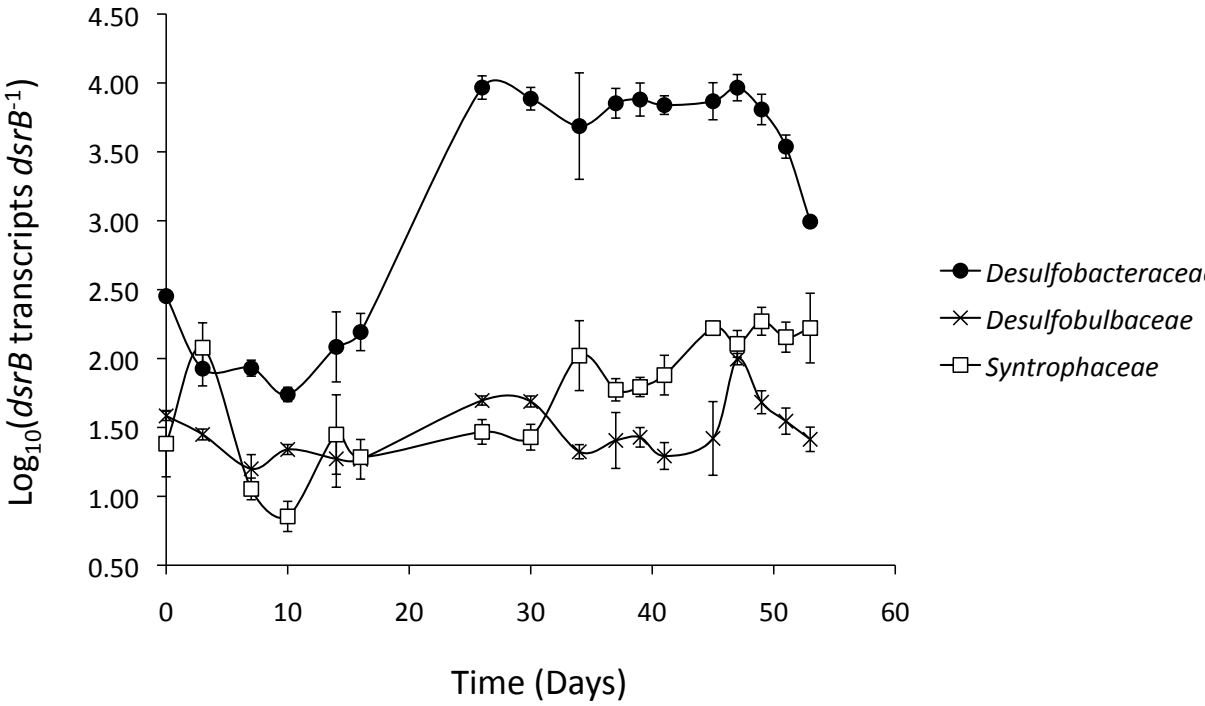

Figure 5. Number of *dsrB* transcripts per copy of *dsrB* for the three major clusters of SRP found in uranium-contaminated groundwater at the Rifle site. Data are means±standard deviation of triplicate determinations.

Table 1. Primers targeting sulfate-reducing prokaryotes (SRP) used in this study.

Primer ID	5'-3' sequence	Target gene	Specificity	References
DSR1F	ACSCACTGGAAGCACG	*dsrAB*	SRP	Wagner et al., 1998
DSR1Fa	ACCCAYTGGAAACACG	*dsrAB*	SRP	Loy et al., 2004
DSR1Fb	GGCCACTGGAAGCACG	*dsrAB*	SRP	Loy et al., 2004
DSR1Fc	ACCCATTGGAAACATG	*dsrAB*	SRP	Zverlov et al., 2005
DSR1Fd	ACTCACTGGAAGCACG	*dsrAB*	SRP	Zverlov et al., 2005
DSR4R	GTGTAGCAGTTACCGCA	*dsrAB*	SRP	Wagner et al., 1998
DSR4Ra	GTGTAACAGTTTCCACA	*dsrAB*	SRP	Loy et al., 2004
DSR4Rb	GTGTAACAGTTACCGCA	*dsrAB*	SRP	Loy et al., 2004
DSR4Rc	GTGTAGCAGTTKCCGCA	*dsrAB*	SRP	Loy et al., 2004
DSR4Rd	GTGTAGCAGTTACCACA	*dsrAB*	SRP	Zverlov et al., 2005
DSR4Re	GTGTAACAGTTACCACA	*dsrAB*	SRP	Zverlov et al., 2005
DSRq1F	CCACAGCAGCCATCAAGCCT	*dsrB*	*Desulfobacteraceae* cluster	This study
DSRq2F	TTGTCCTCTGGGTGCGGTAA	*dsrB*	*Desulfobulbaceae* cluster	This study
DSRq4F	TGCGAGATCCCCACGACCAT	*dsrB*	*Syntrophaceae* cluster	This study
DSRq1R	GTGTAGCAGTTACCGCAGTA	*dsrB*	*Desulfobacteraceae* cluster *Desulfobulbaceae* cluster *Syntrophaceae* cluster	This study

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