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Conservation of the Genes for Dissimilatory Sulfite Reductase from *Desulfovibrio vulgaris* and *Archaeoglobus fulgidus* Allows Their Detection by PCR

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The structural genes for dissimilatory sulfite reductase (desulfoviridin) from *Desulfovibrio vulgaris* Hildenborough were cloned as a 7.2-kbp *Sac*II DNA fragment. Nucleotide sequencing indicated the presence of a third gene, encoding a protein of only 78 amino acids, immediately downstream from the genes for the α and β subunits (*dsvA* and *dsvB*). We designated this protein DsvD and the gene encoding it the *dsvD* gene. The α- and β-subunit sequences are highly homologous to those of the dissimilatory sulfite reductase from *Archaeoglobus fulgidus*, a thermophilic archaeal sulfate reducer, which grows optimally at 83°C. A gene with significant homology to *dsvD* was also found immediately downstream from the *dsrAB* genes of *A. fulgidus*. The remarkable conservation of gene arrangement and sequence across domain (bacterial versus archaeal) and physical (mesophilic versus thermophilic) boundaries indicates an essential role for DsvD in dissimilatory sulfite reduction and allowed the construction of conserved deoxyoligonucleotide primers for detection of the dissimilatory sulfite reductase genes in the environment.

The ability to use sulfate as the terminal electron acceptor is found only in anaerobically living bacteria and archaea. These organisms derive energy for growth from the dissimilatory reduction of sulfate to sulfide by a path that is distinct from that of the assimilatory reduction carried out by a much wider group of living organisms (7, 12). The sulfate-reducing eubacterium *Desulfovibrio vulgaris* has been shown to contain an assimilatory-type sulfite reductase in addition to the dissimilatory sulfite reductase, which is also called desulfoviridin (9, 17). These two reductases have very different subunit compositions: desulfoviridin is an $\alpha_2\beta_2$ tetramer with a molecular mass of 180 kDa, while the assimilatory-type sulfite reductase is a monomeric enzyme with a molecular mass of 24 kDa. The function of the latter enzyme in the metabolism of *D. vulgaris* is currently unknown. The copurification of a third subunit (γ) and evidence for an $\alpha_2\beta_2\gamma_2$-subunit structure that has been presented (10) suggest that the subunit structure of dissimilatory sulfite reductase from *D. vulgaris* may be yet more complex. The *dsvC* gene encoding the γ subunit has been cloned and sequenced (6) but is not linked to those for the α and β subunits. Also, expression of *dsvC* was found to be not coordinately regulated with expression of the genes for the α and β subunits. The precise conditions under which γ binds to the $\alpha_2\beta_2$ core complex, as well as the function of γ in the $\alpha_2\beta_2\gamma_2$ complex, are presently unknown, and we will, therefore, refer to dissimilatory sulfite reductase from *D. vulgaris* as an $\alpha_2\beta_2$ tetramer in the remainder of this article.

Only one dissimilatory sulfite reductase, the enzyme from *Archaeoglobus fulgidus*, a thermophilic archaeal sulfate reducer, has been characterized by molecular genetic analysis (3). The dissimilatory sulfite reductase from *A. fulgidus*, like the enzyme from *D. vulgaris*, is an $\alpha_2\beta_2$ tetramer and binds two sirohemes. It was concluded from sequence comparisons that only the α subunit was likely to bind siroheme. In the present paper, a second sequence for dissimilatory sulfite reductase from a very distant phylogenetic source (*D. vulgaris*) is presented. Comparison of the two sequences allows evaluation of whether sequence elements suggested to bind siroheme are indeed conserved. The unexpectedly high degree of sequence similarity also allows the design of conserved deoxyoligonucleotide primers that can be used for detection of the genes for dissimilatory sulfite reductase by PCR.

MATERIALS AND METHODS

Molecular biology reagents. Deoxyoligonucleotide primers P56 and P57, designed on the basis of the N-terminal sequences of the α and β subunits of *D. vulgaris* desulfoviridin, have been described before (6). Details of plasmids pUC18, pUC19, and pUCBM20 used for cloning are given in Table 1. All radioisotopes were obtained from Amersham, and all restriction and modification enzymes were obtained from Pharmacia.

Cloning and sequencing of the *dsvAB* genes. Southern blotting, with end-labeled P56 or P57 as the probe, indicated the presence of the *dsvAB* genes on a 7.2-kb *Sac*II fragment (6) and a 6.6-kb *Mlu*I fragment. The latter fragment was cloned into plasmid pMM66 (Fig. 1A). Restriction mapping and probing with labeled P56 and P57 indicated the presence of the complete *dsvA* gene but of only the 5′ end of the *dsvB* gene on this plasmid. A labeled *Mlu*I-*Stu*I fragment from pMM66 (Fig. 1A) was used as the probe to clone the 7.2-kb *Sac*II fragment into plasmid pRKS72, which contained both genes in full (Fig. 1B). Recloning into pUC19 gave pSKR72, in which the orientation of the insert relative to the plasmid-borne *lac* promoter was reversed (Fig. 1C). A 4.4-kb *Apa*I-*Sac*II subfragment from this plasmid was cloned into pUC18 to give pAS44 (Fig. 1D).

The insert of pAS44 was sequenced by the dideoxy chain termination method (13) with either Klenow fragment or T7 DNA polymerase. Both sonication and restriction enzyme digestion were used to generate subclones. The sequence data were assembled and analyzed by using the Staden and Genetics Computer Group programs (4, 14, 15).

Analysis of gene expression. Transcription of the *dsv* operon was demonstrated with Northern (RNA) blotting. RNA was isolated from cultures of *Escherichia coli* TG2, transformed with the appropriate plasmids, and from *D. vulgaris* Hildenborough by the hot-phenol method (5). RNA samples were electrophoresed and transferred to Hybond-N hybridization membrane as described elsewhere (1). The blots were probed with ^{32}P-labeled DNA fragments derived from either the *dsvA* or *dsvB* gene (see the legend to Fig. 1).

The expression of the *dsv* operon in *E. coli* was also determined at the level of translation by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and Western blotting (immunoblotting), using 14% (wt/vol) polyacrylamide gels as described by Laemmli (8). Following electrophoresis and transfer of the

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or vector	Genotype, comment(s), and source and/or reference
D. vulgaris Hildenborough	NCIMB 8303; isolated from clay soil near Hildenborough, Kent, United Kingdom (12); source of the *dsv* genes
E. coli TG2[a]	Δ(*lac-pro*) *supE thi hsdM hsdR recA* (F′ *traD36 proAB*$^+$ *lacZ*ΔM15I^q); from T. J. Gibson
pUC18	22
pUC19	22
pUCBM20	Boehringer Mannheim Biochemicals
pMM66	Contains the *dsvA* gene on a 6.6-kb *Mlu*I insert in pUCBM20; this study (Fig. 1)
pRKS72	Contains the *dsvAB* genes on a 7.2-kb *Sac*II insert in pUCBM20; this study (Fig. 1)
pSKR72	Contains the *dsvAB* genes on a 7.2-kb *Sac*II insert in pUC19; this study (Fig. 1)
pAS44	Contains the *dsvAB* genes on a 4.4-kb *Apa*I-*SAc*II insert in pUC18; this study (Fig. 1)

[a] Constructed from *E. coli* JM101 by T. J. Gibson and M. D. Biggin at the Laboratory of Molecular Biology, Medical Research Council Centre, Cambridge, United Kingdom.

polypeptides to nitrocellulose (18), the blots were blocked with gelatin and incubated with a primary antibody against the β subunit of desulfoviridin. Binding of the primary antibody was detected with an alkaline phosphatase-conjugated secondary antibody and immunoblot staining reagents (11).

PCR. Two PCR primers were designed on the basis of nucleotide sequence homologies of the *A. fulgidus* and *D. vulgaris* dissimilatory sulfite reductase genes. The forward primer, P94-F [5′-cctctagaATCGG(A/T)ACCTGGAAGGA(C/T)GACATCAA], and the reverse primer, P93-R [5′-cctctagaGGGCACAT(G/C)GTGTAGCAGTTACCGCA], hybridizing at positions 943 to 968 and 2347 to 2372, respectively, of the nucleotide sequence shown in Fig. 2 were dissolved at a concentration of 50 pmol/μl. For PCR amplification, 2 μl of each primer, 5 to 50 ng of DNA, 2.5 U of *Taq* DNA polymerase, 10 μl of 10× *Taq* polymerase buffer (200 mM Tris-HCl [pH 8.4], 500 mM KCl), 7 μl of 1 M $MgCl_2$, and 8 μl of deoxynucleoside triphosphates (10 mM [each] dTTP, dCTP, dGTP, and dATP) were combined in a final reaction volume of 100 μl. Amplification was carried out for 30 cycles, with each cycle consisting of 1 min at 94°C, 1 min at 60°C, and 1.2 min at 72°C, followed by a final extension at 72°C for 9.9 min. Following the completion of PCR amplification, the PCR product was loaded on a 0.7% high-gelling-temperature (HGT) agarose gel together with molecular size markers (bacteriophage λ DNA digested with *Hin*dIII). The samples analyzed included chromosomal DNAs from oil field bacteria (20, 21), *Rhizobium leguminosarum*, and *Bacillus subtilis*, as well as total community DNAs isolated from oil field production waters (22).

Nucleotide sequence accession number. The sequence reported in this paper has been submitted to GenBank and has been assigned accession number U16723.

RESULTS

Nucleotide sequence and expression of the *dsv* genes. The nucleotide sequence of a portion of the insert of plasmid pAS44, as indicated in Fig. 1E, is shown in Fig. 2. The starting positions of the *dsvA* (nucleotide [nt] 235 to 1548) and *dsvB* (nt 1567 to 2712) genes were unambiguously defined by the N-terminal amino acid sequences determined for the α (AKHAT) and β (AFISS) subunits (6, 10). Evidently, the initiating methionine residue is removed from both sequences following protein synthesis. As in the case of the *Archaeoglobus dsr* operon, the intergenic distance between the 3′ end of the

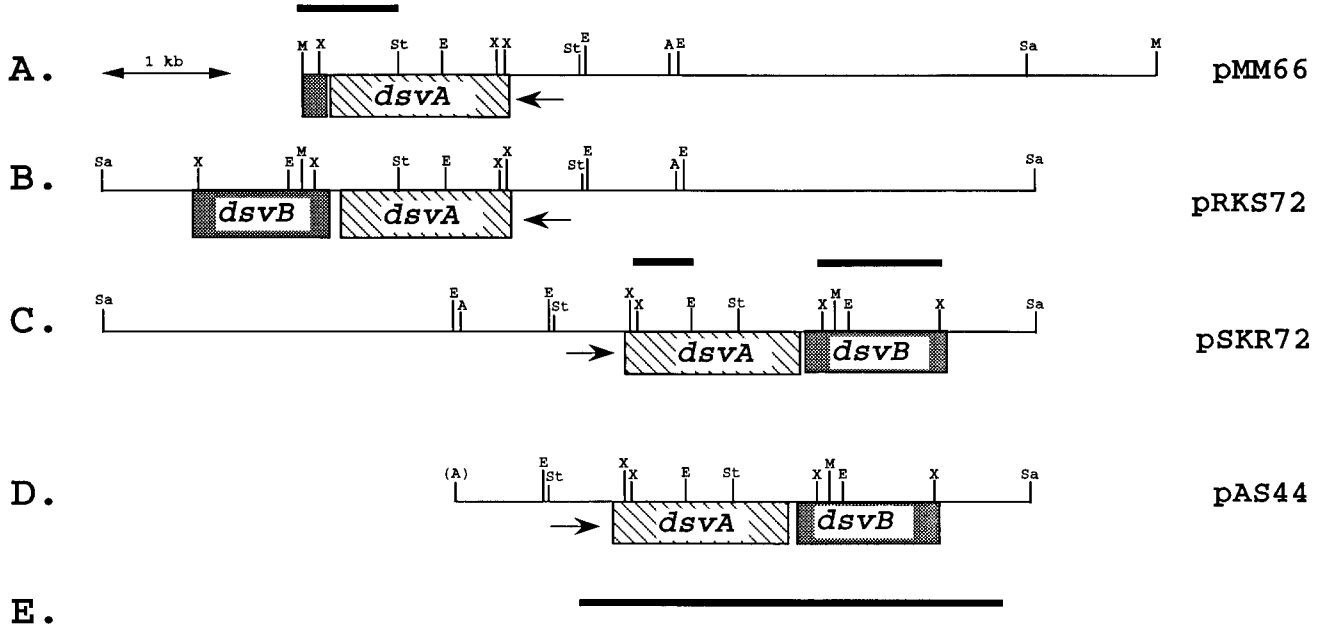

FIG. 1. Restriction map of a region of the *D. vulgaris* Hildenborough genome containing the *dsvAB* genes. The inserts of pMM66, pRKS72, pSKR72, and pAS44 (Table 1) are diagrammed. The *lac* promoter of the vector is always on the left. (A) Map of a 6.6-kb *Mlu*I fragment containing the *dsvA* gene. A 760-bp *Mlu*I-*Stu*I subfragment (▬) was used as the probe for cloning the insert in pRKS72. (B) Map of a 7.2-kb *Sac*II fragment containing the *dsvAB* genes. (C) Same insert as shown in line B in reverse orientation. The 390-bp *Xho*I-*Eco*RI and 860-bp *Xho*I fragments (▬) were used as probes in Northern blotting experiments. (D) Map of a 4.4-kb *Apa*I-*Sac*II subfragment from pSKR72, subcloned into pUC18. (E) Portion of the insert of pAS44 whose sequence is given in Fig. 2. Restriction sites for *Apa*I (A), *Eco*RI (E), *Mlu*I (M), *Sac*II (Sa), *Stu*I (St), and *Xho*I (X) are indicated. The arrows show the direction of transcription from the putative *dsv* promoter. The *dsvD* gene (234 bp), which was discovered by sequencing the insert of plasmid pAS44, is not shown. It is present immediately downstream from *dsvAB*.

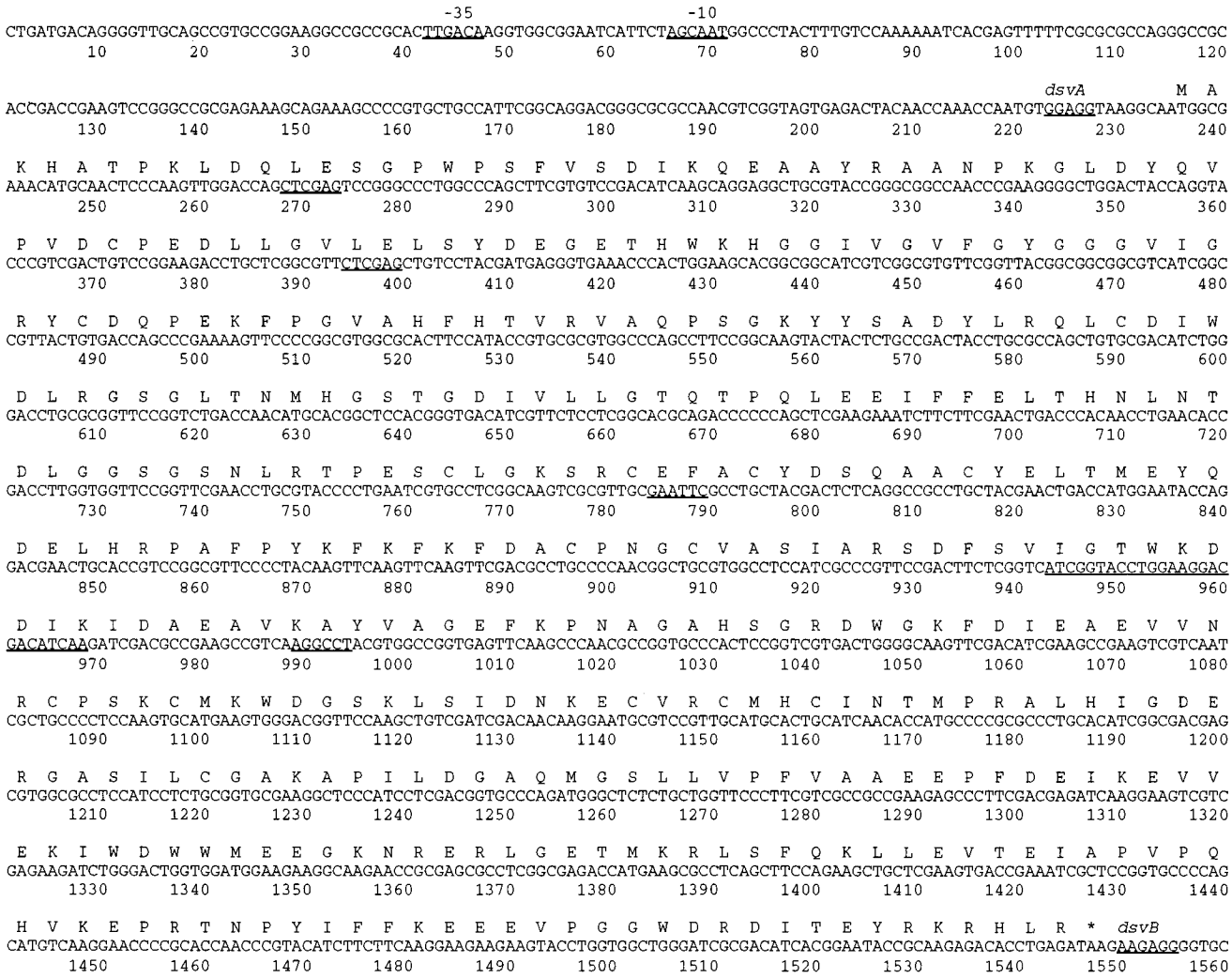

FIG. 2. Nucleotide sequence of the *dsv* operon. The *dsvA*, *dsvB*, and *dsvD* genes are indicated by translation into protein. Other features marked (underlined) in the sequence are the following: at nt 42 to 71, the putative promoter; at nt 223 to 227, the *dsvA* ribosome binding site; at nt 268 to 273, 394 to 399, 1705 to 1710, and 2629 to 2704, *Xho*I sites; at nt 784 to 789 and 1870 to 1875, *Eco*RI sites; at nt 943 to 968, the hybridization locus of the forward PCR primer; at nt 989 to 994, a *Stu*I site; at nt 1550 to 1555, the *dsvB* ribosome binding site; at nt 1761 to 1766, an *Mlu*I site; at nt 2347 to 2372, the hybridization locus of the reverse PCR primer; and at nt 2758 to 2763, the *dsvD* ribosome binding site; and at nt 3041 to 3074, the putative transcription terminator is indicated by half arrows above the sequence. The restriction site positions allow alignment of the sequence with the map in Fig 1D.

dsvA and the 5′ end of the *dsvB* gene is small, only 18 nt. A gene search by codon probability analysis (15) revealed a third open reading frame at nt 2771 to 3007 encoding a protein of only 78 amino acids. Although not reported by Dahl et al. (3), a similar gene, encoding a protein of 77 amino acids, is present in the archaeal sequence starting 41 nt downstream from the 3′ end of the *dsrB* gene. In the *D. vulgaris dsv* operon, the 5′ end of this gene is separated from the 3′ end of *dsvB* by 58 nt. This small gene is named *dsvD*, because the gene for the putative γ subunit of *D. vulgaris* desulfoviridin has already been named *dsvC* (6). The archaeal homolog will be referred to as *dsrD*.

A plausible promoter sequence is present 150 nt upstream from the translational start of the *dsvA* gene, and a putative transcription terminator is found 33 nt downstream from the *dsvD* gene. Transcription initiation and termination at these sites would yield a transcript of 3,000 nt. Northern blotting indicated that *dsv* mRNA isolated from both *E. coli* TG2 transformants and *D. vulgaris* was heterogeneous, with 3,000 nt being near the upper limit. Similarly sized transcripts were observed irrespective of whether a *dsvA*- or *dsvB*-specific fragment was used to probe the blot (data not shown).

Translation of the *dsv* transcript in *E. coli* transformants could be readily demonstrated. Very high levels of both 50- and 40-kDa polypeptides were found in *E. coli* TG2(pAS44) cells (Fig. 3A, lane 6). The sizes of these proteins closely corresponded to the α and β subunits of desulfoviridin purified from *D. vulgaris* Hildenborough (Fig. 3A, lane 7) and were in agreement with the values calculated from the nucleotide sequence (49 and 42 kDa, respectively). Western blotting using a DsvB-specific serum confirmed that the 40-kDa polypeptide was DsvB (Fig. 3B). The 50- and 40-kDa polypeptides were not found in *E. coli* TG2 transformed with vector pUC19, pUCBM20, or pUC18 (Fig. 3, lanes 1 to 3, respectively). Their expression in *E. coli* TG2 depended on the plasmid used for transformation, and the level of expression was relatively low for pRKS72 (Fig. 3, lanes 4, transcription from vector *lac* and insert *dsv* promoters in opposite directions), higher in pSKR72 (Fig. 3, lanes 5, transcription from vector *lac* and insert *dsv*

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      M  A  F  I  S  S  G  Y  N  P  E  K  P  M  A  N  R  I  T  D  I  G  P  R  K  F  D  E  F  F  P  P  V  I  A  K  N  F
AGAAAGATGGCATTCATATCTTCCGGGTACAATCCCGAAAAGCCGATGGCAAACCGTATCACGGACATCGGCCCCCGCAAGTTCGACGAGTTCTTCCCGCCGGTCATTGCCAAGAACTTC
      1570       1580       1590       1600       1610       1620       1630       1640       1650       1660       1670       1680

 G  S  W  L  Y  H  E  I  L  E  P  G  V  L  M  H  V  A  E  S  G  D  K  V  Y  T  V  R  V  G  A  A  R  L  M  S  I  T  H  I
GGTTCGTGGCTGTACCATGAGATCCTCGAGCCCGGCGTGCTCATGCACGTGGCCGAGTCCGGCGACAAGGTGTACACCGTACCGCGTTGGTGCTGCTCGCCTGATGTCGATCACCCACATC
      1690       1700       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800

 R  E  M  C  D  I  A  D  K  Y  C  G  G  H  L  R  F  T  T  R  N  N  V  E  F  M  V  A  D  E  A  S  L  K  A  L  K  E  D  L
CGCGAGATGTGCGACATCGCCGACAAGTACTGCGGCGGTCACCTGCGCTTCACCACGCGTAACAACGTGGAATTCATGGTCGCCGACGAGGCTAGCCTCAAGGCCCTGAAGGAAGACCTC
      1810       1820       1830       1840       1850       1860       1870       1880       1890       1900       1910       1920

 A  S  R  K  F  D  G  G  S  L  K  F  P  I  G  G  T  G  A  G  V  S  N  I  V  H  T  Q  G  W  V  H  C  H  T  P  A  T  D  A
GCCAGCCGCAAGTTCGACGGCGGCTCGCTCAAGTTCCCCATCGGCGGCACCGGCGCTGGCGTGAGCAACATCGTTCACACCCAGGGCTGGGTACACTGCCACACCCCTGCGACCGACGCC
      1930       1940       1950       1960       1970       1980       1990       2000       2010       2020       2030       2040

 S  G  P  V  K  A  I  M  D  E  V  F  E  D  F  Q  S  M  R  L  P  A  P  V  R  I  S  L  A  C  C  I  N  M  C  G  A  V  H  C
TCCGGCCCGGTCAAGGCGATCATGGACGAAGTCTTCGAAGACTTCCAGAGCATGCGCCTTCCCGCTCCGGTTCGCATCTCGCTGGCTTGCTGCATCAACATGTGCGGCGCCGTTCACTGC
      2050       2060       2070       2080       2090       2100       2110       2120       2130       2140       2150       2160

 S  D  I  G  V  V  G  I  H  R  K  P  P  M  I  D  H  E  W  T  D  Q  L  C  E  I  P  L  A  V  A  S  C  P  T  A  A  V  R  P
TCCGACATCGGCGTTGTGGGTATCCACCGCAAGCCCCCGATGATCGACCACGAGTGGACCGACCAGCTGTGCGAAATCCCGCTGGCCGTTGCCTCCTGTCCCACCGCTGCGGTGCGTCCC
      2170       2180       2190       2200       2210       2220       2230       2240       2250       2260       2270       2280

 T  K  L  E  I  G  D  K  K  V  N  T  I  A  I  K  N  E  R  C  M  Y  C  G  N  C  Y  T  M  C  P  A  L  P  I  S  D  G  E  G
ACCAAGCTGGAAATCGGCGACAAGAAGGTCAACACCATCGCCATCAAGAACGAACGCTGCATGTACTGCGGTAACTGCTACACCATGTGCCCCGCGCTGCCCATCTCCGACGGCGAAGGC
      2290       2300       2310       2320       2330       2340       2350       2360       2370       2380       2390       2400

 D  G  V  V  I  M  V  G  G  K  V  S  N  R  I  S  M  P  K  F  S  K  V  V  V  A  Y  I  P  N  E  P  P  R  W  P  S  L  T  K
GACGGCGTGGTCATCATGGTCGGCGGCAAGGTTTCCAACCGCATCTCCATGCCCAAGTTCTCGAAGGTCGTTGTGGCGTACATCCCCAACGAGCCGCCCCGCTGGCCTTCGCTGACCAAG
      2410       2420       2430       2440       2450       2460       2470       2480       2490       2500       2510       2520

 T  I  K  H  I  I  E  V  Y  S  A  N  A  Y  K  Y  E  R  L  G  E  W  A  E  R  I  G  W  E  R  F  F  S  L  T  G  L  E  F  S
ACCATCAAGCACATCATCGAGGTGTACTCGGCCAACGCTTACAAGTACGAGCGTCTGGGCGAGTGGGCTGAGCGCATCGGTTGGGAACGCTTCTTCTCCCTGACCGGCCTCGAGTTCTCG
      2530       2540       2550       2560       2570       2580       2590       2600       2610       2620       2630       2640

 H  H  L  I  D  D  F  R  D  P  A  Y  Y  T  W  R  Q  S  T  Q  F  K  F  *                                                dsvD
CACCACCTCATCGACGACTTCCGCGACCCGGCCTACTACACCTGGCGTCAGAGCACCCAGTTCAAGTTCTAGCCGGCATCGTAACCATAGGGGCCGGTAAACGCCCCGGTTCACTAAAAAG
      2650       2660       2670       2680       2690       2700       2710       2720       2730       2740       2750       2760

         M  E  E  A  K  Q  K  V  V  D  F  L  N  S  K  S  G  S  K  S  K  F  Y  F  N  D  F  T  D  L  F  P  D  M  K  Q  R
GGGTACTTCCATGGAAGAAGCCAAGCAGAAAGTCGTTGACTTCCTGAACTCCAAGTCTGGTAGCAAGAGCAAGTTCTACTTCAACGACTTCACCGACCTGTTCCCCGACATGAAGCAGCG
      2770       2780       2790       2800       2810       2820       2830       2840       2850       2860       2870       2880

  E  V  K  K  I  L  T  A  L  V  N  D  E  V  L  E  Y  W  S  S  G  S  T  T  M  Y  G  L  K  G  A  G  K  Q  A  A  A  E  H  E
TGAAGTGAAGAAGATCCTCACCGCTCTCGTGAACGACGAGGTTCTCGAATACTGGTCCTCGGGCAGCACCACCATGTACGGCCTCAAGGGCGCTGGCAAGCAGGCTGCGGCCGAGCACGA
      2890       2900       2910       2920       2930       2940       2950       2960       2970       2980       2990       3000

  D  *
AGACTAGCCTTCATGCTTAAGGAGCATCGCTCCAGCCAGGAAGCGTATGAACCGCATGGTTCATACGCTCTTTTCTTATGGCCTGCCTTGCACAAGGCAGCGGTAATGCATACGGGAAAC
      3010       3020       3030       3040       3050       3060       3070       3080       3090       3100       3110       3120
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FIG. 2—*Continued.*

promoters in tandem, with promoters separated by 4 kb), and highest in pAS44 (Fig. 3, lanes 6, transcription from vector *lac* and insert *dsv* promoters in tandem, with promoters separated by 1.2 kb).

Sequence similarities. BLAST and FASTA searches with the amino acid sequences of DsvA and DsvB indicated strong similarity to DsrA and DsrB, the α and β subunits of dissimilatory sulfite reductase from *A. fulgidus*. Much weaker similarities to other siroheme-containing proteins (assimilatory sulfite reductase and nitrite reductase) were also found. Since these relationships have already been discussed by Dahl et al. (3), we concentrate in this report on comparing the *dsv* and *dsr* operons.

At the nucleic acid level, despite a large difference in GC content (48.4% for the *A. fulgidus dsr* genes and 60.4% for the *D. vulgaris dsv* genes), the *dsrA-dsvA* and *dsrB-dsvB* pairs of sequences are 58 to 59% identical. In terms of codon usage, the main difference between the *dsr* and *dsv* genes is in the arginine codons, which are almost exclusively AGA or AGG in *A. fulgidus* (38 of 39 codons) and almost exclusively CGT or CGC in *D. vulgaris* (40 of 43 codons). Amino acid sequence alignments are shown in Fig. 4A, B, and D. The percent identities (vertical bars in Fig. 4) and percent similarities (a combination of bars and dots) calculated with the GAP program (11) are, respectively, 56 and 72% for the DsvA-DsrA pair (Fig. 4A), 60 and 77% for the DsvB-DsrB pair (Fig. 4B), and 34 and 60% for the DsvD-DsrD pair (Fig. 4D). As noted already by Dahl et al., there is some homology between the

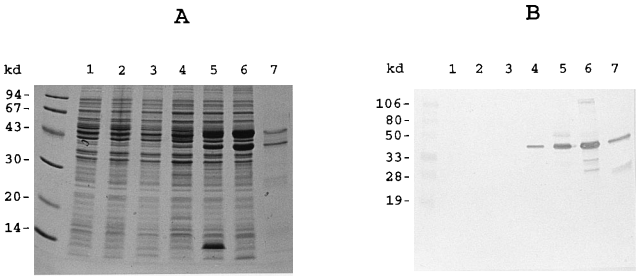

FIG. 3. Expression of DsvA and DsvB in *E. coli* TG2. (A) Coomassie brilliant blue-stained SDS-polyacrylamide gel of total cell extracts. The sizes of protein molecular size markers are indicated on the left. (B) Protein blot incubated with mouse anti-DsvB serum. The sizes of the prestained protein molecular size markers are indicated on the left. The samples used were *E. coli* TG2 transformed with pUCBM20 (lane 1), pUC19 (lane 2), pUC18 (lane 3), pRKS72 (lane 4), pSKR72 (lane 5), and pAS44 (lane 6). A sample of purified desulfoviridin from *D. vulgaris* Hildenborough was run in lane 7.

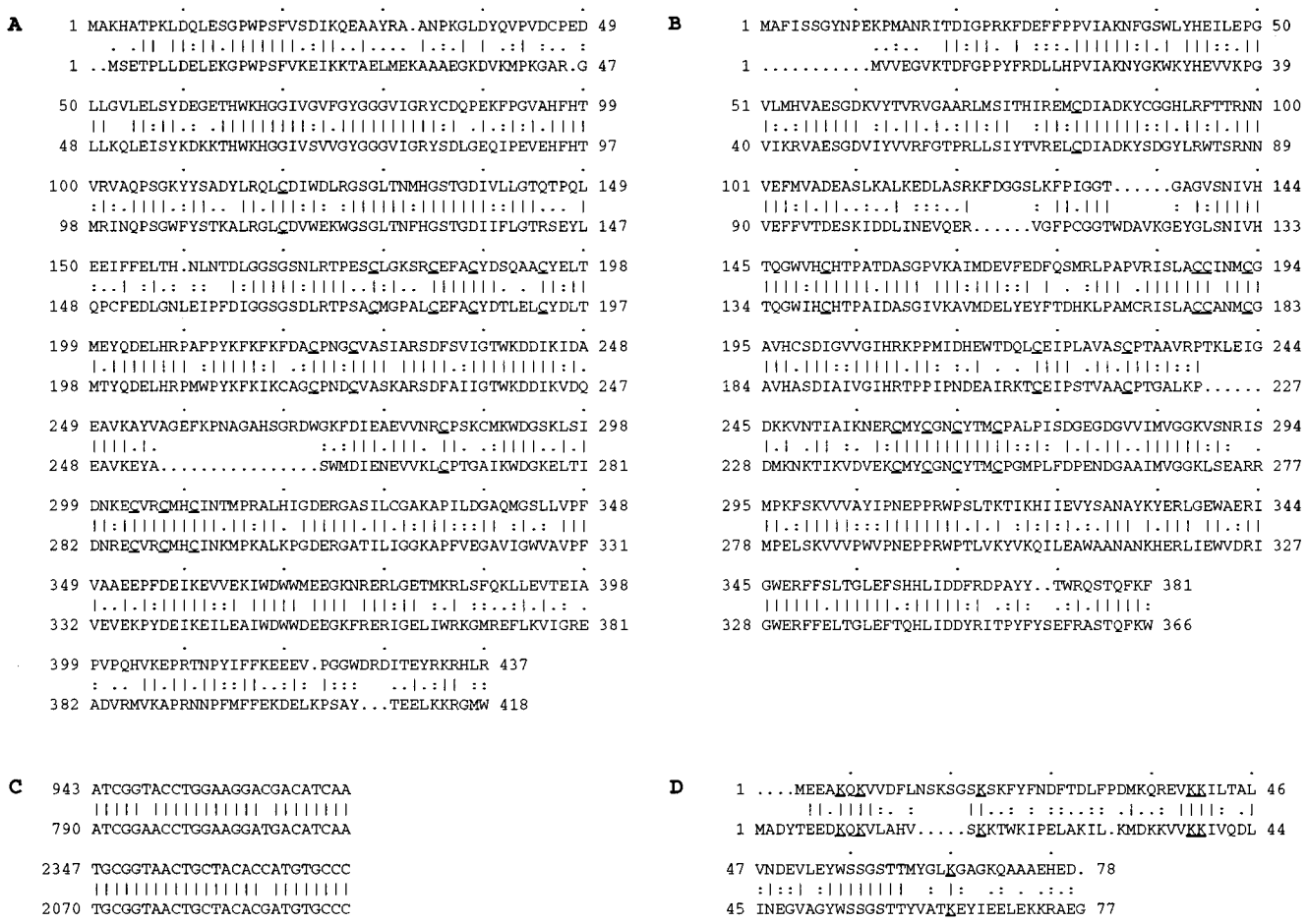

FIG. 4. Sequence comparison of sulfite reductases from the mesophilic eubacterium *D. vulgaris* (upper lines) and the thermophilic archaeon *A. fulgidus* (lower lines). Sequence alignments were made with the GAP program (4). The amino acid sequences of DsvA and DsrA (A), DsvB and DsrB (B), and DsvD and DsrD (D) are aligned. Panel C shows the nucleic acid sequence alignment of two regions used for design of PCR primers. Conserved cysteine residues are underlined in panels A and B. Conserved lysine residues are underlined in panel D. The amino acid sequence numbers (A, B, and D) and the nucleotide sequence numbers (C) are indicated in the margins.

sequences of the α and β subunits, suggesting evolution from a common ancestor. The GAP-calculated values for percent identity and percent similarity, respectively, were 21 and 48% for the DsvA-DsvB pair and 26 and 50% for the DsrA-DsrB pair.

PCR amplification of sulfite reductase genes. The considerable sequence homology between the genes for dissimilatory sulfite reductase of a mesophilic eubacterium and of a thermophilic archaeon that is evident from Fig. 4 allowed the design of deoxyoligonucleotide primers, targeted to hybridize to highly conserved sequences. Two regions, identified in Fig. 4C as starting at nt 943 and 2347 (*D. vulgaris* numbering), showed a particularly high degree of sequence identity with only two and one mismatches per 26 nt, respectively. The forward and reverse primers P94-F and P93-R, whose sequences are given in Materials and Methods, targeted these regions. PCR amplification of plasmid pSKR72 and chromosomal DNA from *D. vulgaris* Hildenborough gave the expected 1.4-kb amplicon (Fig. 5, lanes 1 and 2). A 1.4-kb PCR product was also observed upon amplification of chromosomal DNA from oil field isolates *Lac*1,2 (*D. vulgaris* subsp. *oxamicus*; data not shown), *Lac*3 (*Desulfovibrio desulfuricans* G200; lane 3), *Lac*4 (*Desulfovibrio* sp.; lane 4), *Lac*5 (*Desulfovibrio* sp.; data not shown), *Lac*6 (*Desulfovibrio* sp.; data not shown), *Lac*10 (*Desulfovibrio* sp.; lane 4), *Lac*12 (*Desulfovibrio* sp.; lane 6), *Ace*1 (*Desulfobacter* sp.; lane 8), *Pro*5 (*Desulfobulbus* sp.; lane 9), and *Ben*1 (untyped sulfate-reducing bacterium; lane 10), indicating the presence in these sulfate-reducing bacteria of a sulfite reductase gene similar to that present in *D. vulgaris*. PCR amplification of chromosomal DNA from the aerobic bacteria *B. subtilis* (Fig. 5, lane 7) and *R. leguminosarum* (lane 12) yielded no 1.4-kb PCR product. Finally, the presence of dissimilatory sulfite reductase genes in environmental samples could also be demonstrated by amplification of total-community DNAs isolated directly from oil field production waters (lane 11).

DISCUSSION

Dissimilatory sulfate reduction by which bacteria produce copious amounts of sulfide as the result of respiratory metabolism can significantly affect human-induced and natural environmental processes. For instance, in the oil and gas industry, the gradual increase in sulfide concentration in the oil or gas produced can become a serious problem. For fields at moderate temperatures, the involvement of mesophilic, sulfate-reducing eubacteria (*Desulfovibrio* spp., *Desulfobacter* spp., and others) in this process is well documented (2, 19). The weight

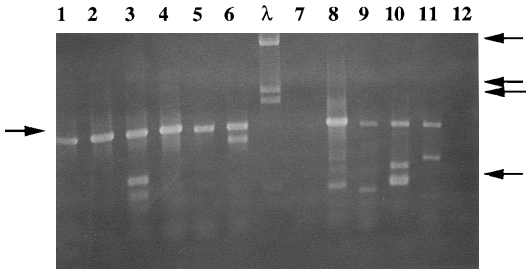

FIG. 5. PCR amplification of the genes for dissimilatory sulfite reductase yields the expected 1.4-kb product (→). The amplification products were electrophoresed on a 0.7% (wt/vol) HGT agarose gel. Following electrophoresis and staining with ethidium bromide, the gel was photographed. Lane 1, plasmid pRKS72; lane 11, total-community DNA from oil field production water containing sulfate-reducing bacteria; lane λ, bacteriophage λ DNA digested with *Hin*dIII; the positions of the 4.4-, 2.3-, 2.0-, and 0.56-kb markers are shown (←). The remaining lanes contain chromosomal DNAs. Lane 2, *D. vulgaris* Hildenborough; lane 3, *Lac*3 (*D. desulfuricans* G200); lane 4, *Lac*4 (*Desulfovibrio* sp.); lane 5, *Lac*10 (*Desulfovibrio* sp.); lane 6, *Lac*12 (*Desulfovibrio* sp.); lane 7, *B. subtilis*; lane 8, *Ace*1 (*Desulfobacter* sp.); lane 9, *Pro*5 (*Desulfobulbus* sp.); lane 10, *Ben*1 (untyped isolate); lane 12, *R. leguminosarum.*

of evidence on the question whether sulfide formation in high-temperature fields, such as those found in the Alaska North Slope or in the North Sea which have resident temperatures of 100 to 110°C, is biogenic or abiogenic was recently tilted in favor of the former explanation with the discovery of hyperthermophilic archaea in production fluids from these fields (16). *Archaeoglobus*, *Thermococcus*, and *Pyrococcus* spp. were among the species reported present. We have demonstrated in this study a remarkable degree of conservation of dissimilatory sulfite reductase, the terminal redox enzyme that catalyzes the reduction of sulfite to sulfide, between the mesophilic eubacterium *D. vulgaris* and the thermophilic archaeon *A. fulgidus*. In view of the distant phylogenetic origins of these two sources, the observed degree of conservation offers the enticing possibility that dissimilatory sulfite reductases are highly conserved throughout the microbial world. The use of conserved deoxyoligonucleotide primers (Fig. 4C) allows this issue to be settled by rapid PCR assays, which can demonstrate whether a dissimilatory sulfite reductase similar to that found in *Desulfovibrio* and *Archaeoglobus* spp. is present in a given microorganism. Our first steps along this path were encouraging (Fig. 5) and have indicated the presence of this dissimilatory sulfite reductase in a variety of sulfate-reducing bacteria. These studies should be extended to other classes of bacteria thought to contain the genes for dissimilatory sulfite reductase. It is important to definitively answer this question, because the potential or actual production of large amounts of sulfide in an environment can potentially be analyzed by PCR assays, provided that all dissimilatory sulfate-reducers are responsive to the selected primer set. This would allow monitoring of biological sulfide production in diverse environmental processes, e.g., oil field souring, metal corrosion, and concrete corrosion of sewer pipes, as well as removal of heavy metals from acid mine drainage effluents in wetlands.

Biophysical studies on purified dissimilatory sulfite-reductases have indicated several distinguishable types (3). All have an $\alpha_2\beta_2$-subunit structure and a molecular weight of 170,000 to 220,000 and usually contain two sirohemes as well as iron-sulfur clusters, possibly as many as four of the Fe_4S_4 type (3). Dahl et al. (3) indicated that on the basis of homology with other siroheme-containing redox enzymes (e.g., assimilatory sulfite reductase and nitrite reductase), which all contain a siroheme-binding sequence, C-X_5-C-X_n-C-X_3-C, the α subunit

```
Asr      83 CPGTAVCRFG LQDSLGIGVA IEEEYVGH-- --DFPAKVKF GISGCPFCC
DsvA    165 CLGKSRCEFA CYDSQAACYE LTMEYQDELH RPAFPYKFKF KFDACPNGC
DsrA    167 CMGPALCEFA CYDTLELCYD LTMTYQDELH RPMWPYKFKI KCAGCPNDC
DsvB    135 TQGWVHCHTP ATDASGPVKA IMDEVFEDFQ SMRLPAPVRI SLACCINMC
DsrB    124 TQGWIHCHTP AIDASGIVKA VMDELYEYFT DHKLPAMCRI SLACCANMC
Sir         C     C                                       C   C
```

FIG. 6. Alignment of putative siroheme-binding regions. Asr, assimilatory sulfite reductase of *D. vulgaris* (17); DsvA and DsrA, α subunits of dissimilatory sulfite reductase of *D. vulgaris* and *A. fulgidus*, respectively; DsvB and DsrB, β subunits of dissimilatory sulfite reductase of *D. vulgaris* and *A. fulgidus*, respectively. The residues conserved in Asr, DsvA, and DsrA have been underlined. The residues conserved in all five sequences are in boldface type and underlined. The consensus siroheme-binding region C-X_5-C-X_n-C-X_3-C is also indicated (Sir).

is most likely to bind siroheme, with no siroheme binding to the β subunit. Dot matrix comparison of the amino acid sequences of the α subunit of *D. vulgaris* dissimilatory sulfite reductase and of *D. vulgaris* assimilatory sulfite reductase indicated that sequence similarity was restricted to the putative siroheme-binding regions. The alignment of these regions (shown in Fig. 6) indicated 12 of 49 residues (24%) to be identical. The best alignment of the β-subunit sequences with the putative siroheme-binding region of dissimilatory sulfite reductase had a much smaller degree of sequence identity (Fig. 6, 6 of 49 residues [12%]). This lower degree of sequence identity, together with the fact that the first Cys residue in the siroheme-binding consensus sequence C-X_5-C-X_n-C-X_3-C is missing from the β-subunit alignments (Fig. 6), confirms the conclusion of Dahl et al. that only the α subunit binds siroheme. With respect to other iron-sulfur clusters, it should be noted that alignment of the α- and β-subunit sequences indicated the presence of 11 conserved cysteine residues in both subunits (Fig. 4A and B). After subtracting the four residues of the α subunit thought to be involved in siroheme iron-sulfur cluster binding, 36 conserved cysteine residues remain per $\alpha_2\beta_2$ tetramer. This provides more than sufficient thiol ligands for coordination of a suggested additional four Fe_4S_4 clusters, which requires 16 cysteine residues. Residues 266, 285, 288, and 291 in the α subunit and 219, 240, 243, and 246 in the β subunit of *A. fulgidus* dissimilatory sulfite reductase are all strong candidates, since they have all been conserved in the *D. vulgaris* sequences (Fig. 4A and B).

The function of DsvD is at present an enigma. Because Pierik et al. (10) reported the binding of an 11-kDa polypeptide to the $\alpha_2\beta_2$ tetramer of *D. vulgaris* (which has been named the γ subunit or DsvC, encoded by the *dsvC* gene [6]), Dahl et al. (3) screened their purified preparations for the presence of a low-molecular-weight protein but none was found. Thus, DsvD and DsrD do not copurify with dissimilatory sulfite reductase in either *D. vulgaris* or *A. fulgidus*. The conserved length (78 versus 77 residues) and sequence (34% identity; Fig. 4D) imply an essential role of this protein in dissimilatory sulfite reduction. The absence of Cys residues makes an electron transfer function mediated by iron-sulfur clusters unlikely. The high content and significant conservation of Lys residues (6 of a total of 10 and 14 residues in DsvD and DsrD, respectively) could indicate a function as an anion (e.g., sulfite)-binding protein, and this possibility should be tested.

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