Supporting Information

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SI Methods

Strains and Culture Conditions. *Desulfovibrio vulgaris* Hildenborough (ATCC 29579) was obtained from T. Hazen (Oak Ridge National Laboratory, Oak Ridge, TN). A clone of *D. vulgaris* Hildenborough (D1) was isolated by plating as described previously (1). A spontaneous nalidixic acid-resistant mutant (D2) was subsequently isolated from the D1 clonal line (1). *Methanococcus maripaludis* S2 was obtained from J. Leigh (University of Washington Seattle, Seattle, WA). As described previously (1), a clone of *M. maripaludis* (M1) was isolated by plating. A spontaneous neomycin-resistant mutant (M2) was subsequently isolated from the M1 clonal line. All cultures were incubated at 37 °C.

D. vulgaris, *M. maripaludis*, and cocultures of the two species were propagated as described previously (1). Cocultures were propagated in Balch tubes containing ∼20 mL of coculture medium A (CCMA) (2) with a 80% N_2:20% CO_2 headspace. CCMA contains lactate as the electron donor, several salts (NaCl, $MgCl_2$, $CaCl_2$, NH_4Cl, KCl, and KH_2PO_4), bicarbonate as a buffer, cysteine and sodium sulfide as reducing agents, Thauer's vitamins, trace minerals, and the pH and the redox-potential indicator resazurin. A detailed recipe is available in refs. 1–3. Pure cultures of *D. vulgaris* were propagated similarly, except that the CCMA was amended with 30 mM Na_2SO_4 or 5 mM Na_2SO_3 depending on whether we were measuring sulfate respiration or isolating *D. vulgaris* populations that could include both sulfate respiration (SR)-negative and SR-positive subpopulations. *M. maripaludis* was propagated in pure culture in Balch tubes with 5 mL of CCMA lacking lactate and amended with 0.82 g/L sodium acetate, and 1 g/L casamino acids. These tubes were incubated in a horizontal position with shaking at 300 rpm after the headspace was pressurized to 40 psi with 80% H_2:20% CO_2.

Propagation of Evolution Lines. The evolution experiment was initiated and propagated as described previously (1). Four starting cocultures were established from all four combinations of *D. vulgaris* (D1, D2) and *M. maripaludis* (M1, M2) ancestral clones. Three replicates of each ancestral pairing were propagated with and without constant shaking at 300 rpm in a horizontal or vertical position, respectively. A volume of 0.2 mL of culture fluid was transferred once a week to 20 mL of CCMA for 152 transfers, or 1,003 generations. Subsamples of cultures were periodically mixed with glycerol to a final concentration of 20% (vol/vol) and stored at −80 °C for archive. U1–U3 and H1–H3 descended from clones D2 and M2; U4–U6 and H4 descended from clones D1 and M1; U7–U9 and H5–H7 descended from clones D2 and M1; U10–U12 and H8–H10 descended from clones D1 and M2.

Sequencing and Analysis of Evolved Cocultures. Biomass samples were collected from 50-mL cocultures grown to stationary phase. The coculture samples were centrifuged at 15,000 × *g* for 10 min to pellet the biomass and the supernatant was removed. The DNA was extracted from the remaining biomass sample using the Masterpure Total DNA Purification kit (Epicentre) following the manufacturer's protocol.

The extracted DNA was sequenced on the Illumina MiSeq platform (Illumina) for paired-end reads (2 × 250 bp). DNA libraries were prepared using Nextera DNA library preparation kit (Illumina) according to the protocol of the manufacturer. Briefly, 50 ng of DNA (20 μL at 2.5 ng/μL) was fragmented using 5 μL of Tagment DNA enzyme with 25 μL of Tagment DNA buffer. Tagmentation reactions were performed by incubation at 55 °C for 5 min followed by purification of the tagmented DNA using the Zymo Clean and Concentrator-5 kit (Zymo Research). Purified DNA was eluted from the column with 25 μL of resuspension buffer. Illumina adapters and index were added to the purified tagmented DNA (20 μL) by limited-cycle PCR (five cycles) amplification with index 1 and 2 primers in a 50-μL reaction according to the Nextera protocol. Amplified DNA was purified using 30-μL AMPure XP beads (Beckman Coulter). The fragment size distribution of the tagmented DNA was analyzed using a 2100 Bioanalyser with a High Sensitivity DNA assay kit (Agilent Technologies). DNA libraries were normalized to 2 nM, pooled in equal volumes.

Sample libraries for sequencing were prepared according to the MiSeq Reagent Kit Preparation Guide (Illumina). Briefly, the pooled sample library (2 nM) was denatured by mixing 10 μL of the library and 10 μL of 0.2 M fresh NaOH and incubated 5 min at room temperature. A volume of 980 μL of chilled Illumina HT1 buffer was added to the denatured DNA and mixed to make a 20 pM library. The 20 pM library was further adjusted to reach the desired concentration for sequencing; for example, 625 μL of the 20 pM library was mixed with 375 μL of chilled Illumina HT1 buffer to make a 12.5 pM library. The library for sequencing was mixed with about 10% (vol/vol) PhiX library of the same concentration (as suggested by Illumina technical support).

A 500-cycle version 2 MiSeq reagent cartridge (Illumina) was thawed for 1 h in a water bath at room temperature, inverted 10 times to mix the thawed reagents, and stored at 4 °C or on ice until use. Sequencing was performed for 251, 12, and 251 cycles for forward, index, and reverse reads, respectively on MiSeq.

To determine the mutations within each line, the resulting raw Illumina sequences were first trimmed for quality using Brtim with the following parameters (-q -S -o -w 5 -a 20) (4). The quality trimmed sequences were then aligned to the reference *D. vulgaris* (NC_002937, NC_005863) and *M. maripaludis* (NC_005791) published genomes using the breseq pipeline (5). The Genome Analysis Toolkit (GATK) (www.ncbi.nlm.nih.gov/pubmed?term=21478889) pipeline for variation discovery was used as an additional validation. Briefly, reads were first aligned to the reference genome using bwa (www.ncbi.nlm.nih.gov/pubmed/19451168) (-M -t 4 –R). The resulting alignment SAM files were converted to BAM files and sorted. BAM files were marked for duplicates using Picard Tools (http://picard.sourceforge.net/), and local realignment around indels was performed to identify the most consistent placement of reads relative to the indels. Variant calling was performed either by using GATK UnifiedGenotyper or Varscan (www.ncbi.nlm.nih.gov/pubmed/22300766). The default parameters were used for UnifiedGenotyper, whereas for Varscan parameters were –min-coverage 20 –min-var-freq 0.2. The resulting variants were annotated using SnpEff tools (www.ncbi.nlm.nih.gov/pubmed/22728672).

Generation of End-Point Dilution Lines. Eight evolved cocultures (U8, U9, U12, H2, H4, H6, H8, and H10) were grown from 20% (vol/vol) glycerol stock storage in 20-mL CCMA medium under an 80% N_2:20% CO_2 atmosphere. Once cocultures had reached stationary growth phase (0.5 OD_{600nm}), 200 μL of the coculture was then transferred to 20 mL of fresh CCMA medium and incubated until it entered late-exponential phase. It was then used to make two 10,000-fold diluted cultures in fresh CCMA. These two diluted cultures were then used to found 10 replicate dilution series, each consisting of six 10-fold serial dilutions. This procedure resulted in 10 lines per evolved coculture that had been diluted by 1×10^{11} in CCMA. These were incubated at

37 °C and monitored for growth for 10 d. The greatest dilution of culture that showed growth in each line was then used as the source for a second round of serial dilutions to create simplified communities that were used for further analyses. The cultures were then mixed with glycerol to a final concentration of 20% (vol/vol) and stored at −80 °C.

To determine the sulfate respiration capability of *D. vulgaris* populations in the end-point dilutions (EPDs), 200 μL of each EPD culture was added to a fresh 10-mL Balch tube containing CCMA with sulfate and 2.5 μg of puromycin per mL to inhibit the *M. maripaludis* growth. The cultures were then incubated at 37 °C and monitored for 4 wk to determine the capacity to initiate growth. Cultures that grew were then transferred into a new tube of CCMA with sulfate and puromycin and their growth was monitored to confirm the capacity for sulfate respiration. The presence or absence of mutations in these EPD lines was tested with Sanger sequencing.

Sanger Sequencing to Identify and Confirm Mutations. Primers were designed to target regions of sequence encompassing mutations in *aps*A, *aps*B, *sat*, and *dsr*C observed in the evolved populations (Table S3). The target region for each gene was amplified from DNA extracted from 10 EPDs from evolved cocultures H6, U9, H10, H2, and H8. The PCR product was purified with Zymo Clean and Concentrator-5 (Zymo Research) and sent to Eurofin MWG Operon for Sanger sequencing. The presence or absence of mutations was determined by viewing the sequencing chromatograms in Sequencher, version 4.9 (Gene Codes). To determine whether mutations in *sat*, *aps*A, *aps*B, and *dsr*C were present in the nine evolved lines that were not resequenced with Illumina, each gene was amplified by PCR from genomic DNA of each population using the same primers as described above. PCR products were cleaned with the Wizard DNA Clean-Up System (Promega; part A7280) and chromatograms were viewed and analyzed using GENtle (version 1.9.4; gentle.magnusmanske.de).

Construction of Mutants. ***Sat deletion mutant.*** The *sat* (DVU1295) deletion mutant (JW9271; see Table S6 for strains and plasmids used in this study) was constructed in a similar manner as described previously (6). In short, two plasmids were made to accomplish this: pMO9268 and pMO9270. The plasmid pMO9268 ("marker-exchange plasmid") is a suicide vector that contains the pUC origin of replication, the spectinomycin resistance gene, the 1,126-bp region upstream of the *sat* gene, a two-gene operon containing the kanamycin resistance gene [*aph(3′)-II*] and the uracil-phosphoribosyltransferase gene (*upp*; DVU1025) from *D. vulgaris* Hildenborough, and a 1,101-bp region downstream of the *sat* gene. A successful transformation of the marker-exchange plasmid into a strain of DvH lacking the *upp* gene [JW710, resistant to 5-fluorouracil ($5FU^r$)] will replace the *sat* gene (DVU1295) with the kanamycin resistance and *upp* genes via a double-homologous recombination event. This would result in a transformant resistant to kanamycin (or G418, geneticin; $G418^r$) and sensitive to 5FU.

Transformation was accomplished by electroporation (1,500 V, 250 Ω, 25 μF; 1-mm cuvette) with an ECM 630 electroporator (BTX). Cells were allowed to recover overnight in MOYLS3 medium (7) without selection. Three different volumes (10, 100, and ∼890 μL) were placed into empty Petri dishes and 25 mL of molten MOYLS3 agar (cooled to 50 °C) containing 400 μg of G418 per mL was poured into the Petri dishes. The cells in molten agar were mixed briefly by swirling, and the medium allowed to cool and solidify. The plates were incubated for 3 d at 37 °C in an anaerobic box containing an anaeropack (Mitsubishi Gas Company of America, distributed by ThermoFisher). Putative transformants were screened with MOYLS3 agar plates for resistance to G418, sensitivity to 5FU (40 μg/mL), and sensitivity to spectinomycin (50 μg of spectinomycin per mL). A plate without antibiotics was also inoculated as a positive control for growth. Colonies showing the expected inhibitor phenotype were grown overnight in 0.5 mL of MOYLS3, subcultured into 5 mL of MOYLS3, grown overnight, and then freezer stocks were prepared [with a final concentration of glycerol of 10% (vol/vol)]. One of these marker-exchange isolates (JW9269) was electroporated with pMO9270 ("markerless deletion plasmid"), recovered, and plated similarly as above (with 5FU in place of G418). The pMO9270 plasmid contains the same elements as the pMO9268 plasmid except that it lacks the kanamycin resistance and *upp* genes. The isolates were screened as above, except that transformants were selected that were sensitive to G418 and resistant to 5FU. To verify the strains by Southern blot, genomic DNA from putative markerless deletion isolates (JW9271), the marker-exchange strain (JW9269), and the JW710 parent strain were digested with the restriction enzyme BglI (NEB) and subjected to gel electrophoresis [0.8% (wt/vol) agarose]. The DNA was transferred to Zeta-Probe (Bio-Rad) and hybridized with a radioactively labeled DNA probe (made with Prime-it RmT Random Primer Labeling Kit; Agilent) from the upstream region of the *sat* gene as the template. Bands of distinct sizes for each strain corresponded to predicted sizes. Additionally, the strain was tested for growth on sulfate as the electron acceptor to check the expected SR-negative phenotype.

The pMO9268 and pMO9270 plasmids were constructed by sequence and ligation-independent cloning (SLIC) (8). The PCR products were obtained by amplification with Herculase II (Life Technologies) with the primers (Integrated DNA Technologies) in Table S2. Template DNA included genomic DNA from JW710 for upstream and downstream regions, pCR8/GW/TOPO plasmid (Life Technologies) for the spectinomycin resistance gene and pUC ori, and pMO746 (9) for the kanamycin resistance and *upp* genes. The PCR products were cleaned with the Wizard SV gel and PCR cleanup system (Promega). The PCR products were quantified by a NanoDrop ND-1000 spectrophotometer (ThermoFisher) and mixed together in equal molar ratios (∼400 fmol each) to a final volume of 20 μL. The DNA mixture was treated with T4 DNA polymerase (NEB) and incubated at room temperature for 30 min. The reaction was stopped by addition of 2 μL of 10 mM dCTP (ThermoFisher). An aliquot of the treated DNA (5 μL) was added to 50 μL of competent *Escherichia coli* cells (silver-efficiency α-select; Bioline) and incubated on ice for 30 min. The cells were transformed according to instructions of the manufacturer of the competent cells and allowed to recover in 0.25 mL of SOC (7) at 37 °C for 1 h. The recovered *E. coli* cells were plated on LC agar (7) plates containing the appropriate antibiotic (kanamycin for the marker-exchange plasmid and spectinomycin for the markerless deletion plasmid). Putative constructs were sequenced (DNA Core, University of Missouri) to confirm the correct sequences for the upstream and downstream regions.

Aps deletion mutant. Construction of the *apsBA* (DVU0846-7) deletion mutant (JW9259) was accomplished in a similar manner as the *sat* deletion mutant. The plasmids pMO9256 (marker-exchange) and pMO9258 (markerless deletion) and intermediate strain JW9257 (marker-exchange) were also made. Upstream region of *apsBA* is 715 bp, and the downstream region is 700 bp. Restriction enzyme *AatII* (Fermentas) was used for Southern blot verification. See Table S3 for corresponding primers.

QmoABC deletion mutant. Construction of the *qmoABC* (DVU0848-50) deletion mutant (JW9263) was accomplished in a similar manner as the *sat* deletion mutant. The plasmids pMO9260 (marker-exchange) and pMO9262 (markerless deletion) and intermediate strain JW9261 (marker-exchange) were also made. Upstream region of *qmoABC* is 977 bp, and the downstream region is 714 bp. Restriction enzyme *XcmI* (NEB) was used for Southern blot verification. See Table S3 for corresponding primers.

Statistical Analyses. All statistical analyses were performed using Proc MIXED or Proc GLM in SAS, version 9.3 (10). To examine the effects of 1,000-generation *D. vulgaris*, *M. maripaludis*, or both (composition) on growth rate and yield of cocultures, and test whether these effects differed when cocultures evolved in the uniform or heterogeneous environments (EvolEnv) (Fig. 1 and Table S1), we used the following statistical model: Growth rate or yield of D_EM_E, D_EM_A D_AM_E D_AM_A = Evolenv + Block + Composition + EvolEnv*Composition + Coculture(EvolEnv) + Composition*coculture(EvolEnv). Coculture(EvolEnv) and Composition*Coculture(EvolEnv) were both treated as random factors because the 22 evolved cocultures represent only a fraction of the potential evolutionary outcomes that could occur in the evolution conditions. The effect Comp*coculture(EvolEnv) served as the error term for testing the fixed effects of EvolEnv, Block, Composition, and Composition*EvolEnv when coculture growth rate was the response variable. However, the covariance for this parameter was "0" when the same model was used to evaluate coculture yield. Thus, experimental error was used to test the effects of the fixed effects for evaluations of coculture yield.

We used Proc GLM to test the effects of several *D. vulgaris* strains (ancestral *D. vulgaris*, evolved SR-positive and SR-negative *D. vulgaris* from coculture H2, and mutants of *D. vulgaris* strain JW710 containing deletions in either *sat*, *aps*AB, or *qmo*ABCD) paired with one of three *M. maripaludis* strains (ancestral *M. maripaludis*, evolved *M. maripaludis* population from SR-positive EPD or from SR-negative EPD from coculture H2) (Fig. 3 and Table S4). The statistical fixed-effects ANOVA model was as follows: coculture growth rate or yield = *D. vulgaris* + *M. maripaludis* + *D. vulgaris* × *M. maripaludis*. Much of the variation in the model appeared to be driven by differences between cocultures with evolved *D. vulgaris* populations and those *D. vulgaris* that did not evolve. Thus, to test the effects of the mutations more directly, we repeated the same statistical tests with only cocultures containing *D. vulgaris* strain JW710 or the mutants made from that strain.

Using Proc GLM, we tested whether there was variation in growth among evolved cocultures H2, H6, and U9 and several specific EPD cocultures that were derived from them (Table S5, U9 and H6, two SR-positive EPD and two SR-negative EPD each; H2, two SR-negative and one SR-positive EPD) . Ancestral cocultures were significantly different from all evolved cocultures, and comparisons to their growth were not part of the hypotheses tested. We therefore did not include them in the statistical models. The following ANOVA model was used: Coculture growth rate or yield = Evolved coculture + EPD line(Evolved coculture). Because each EPD culture came from a specific coculture, the effect of "EPD line" was nested within the effect "evolved coculture." We used contrasts to determine, for each evolved coculture, whether the specific SR-positive and SR-negative EPD cultures had the same mean growth rate or yield. The coefficients for contrasts with U9 and H6 were 0 1 1 −1 −1 (the evolved coculture from which the EPDs were derived was not tested in the contrast), and for H2 the coefficients were 0 2 −1 −1. These coefficients essentially test whether the sum of means for SR-positive cocultures differs from the sum of means for SR-negative cocultures.

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10. SAS Institute (2010) SAS (SAS Institute, Cary, NC), Version 9.3.

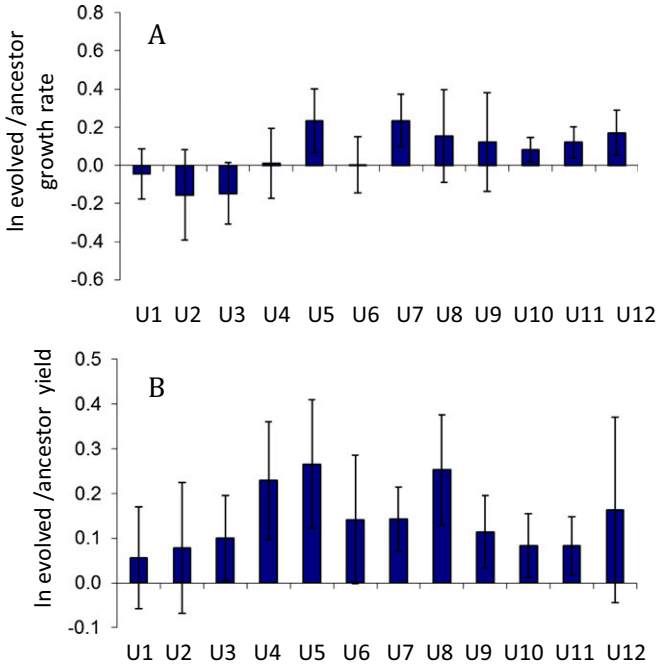

Fig. S1. Growth, relative to the ancestor, of SR-positive *D. vulgaris* populations that have evolved in the uniform environment for 1,000 generations. These populations were enriched from their native community in media containing sulfate and puromycin. Growth rate (*A*) and yield (*B*) of these populations relative to the ancestor are shown below. Error bars indicate 95% confidence intervals for the mean of four replicates.

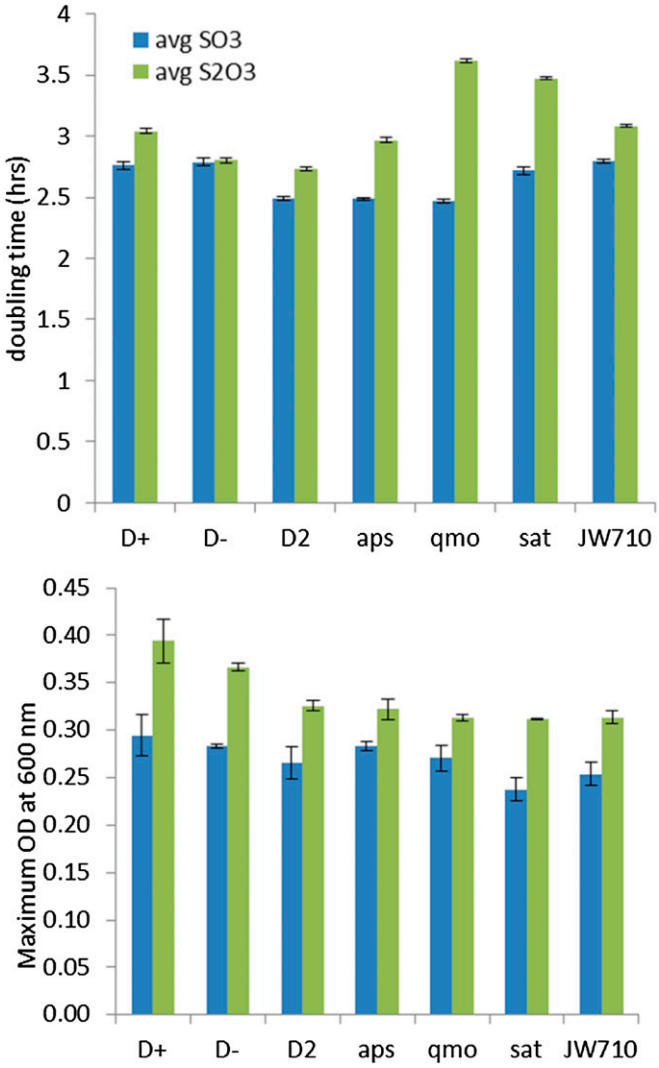

Fig. S2. Yield and doubling time of mutants respiring sulfite or thiosulfate with lactate as the electron donor. D+ and D− are SR-positive and SR-negative clones of *D. vulgaris* that were isolated from coculture H2. D2 is the ancestral *D. vulgaris* and JW710 is the background strain used to make the markerless deletions in the *aps*AB, *qmo*ABC, and *sat* genes.

Table S1. Mixed-model ANOVAs testing the effects of *D. vulgaris* and *M. maripaludis* evolution on coculture growth improvements

Source of variation	Num df	Den df	*F*	*P*
Growth rate				
Evolution environment	1	20	0.03	0.8609
Block	1	26.2	0.19	0.6647
Composition	3	60.2	348.3	<0.001
EvolEnv*Comp	3	60.2	7.59	0.0002
Yield				
Evolution environment	1	20.1	1.58	0.2231
Block	1	321	1.49	0.2230
Composition	3	321	474.0	<0.0001
EvolEnv*Comp	3	321	7.17	0.0001

Evolution environment tests whether populations from the heterogeneous or uniform evolution environments had different effects on coculture growth. Block tests the effects of variation between replications of the experiments. Composition tests whether D_EM_E, D_EM_A D_AM_A, or D_AM_E cocultures have different growth rates or yield, and EvolEnv*Comp tests whether the effects of composition depend on the evolution environment.

Table S2. Description of mutations in genes required for sulfate reduction in *D. vulgaris*

Evolved coculture	*sat*	*aps*A	*aps*B	*dsr*C	1999*	0916[†]
U1			92[‡] fs−1[¶]	233 A:T[§] misS		
U2[#]			175[‖] C:T nonS		nt**	nt
U3					nt	nt
U4	653 G:T nonS	421 C:T nonS		17 A:G misS	T:G 910 misS	
U5		214 C:T nonS			nt	nt
U6				17 A:C misS	nt	nt
U7					nt	nt
U8						298 G:T misS
U9	142 fs−1		16 fs−1	301 C:G misS		
U10	142 fs−1		16 fs−1	301 C:G misS	nt	nt
U11			1 A:C syn	232 A:G misS		
U12	593 A:C misS	421 C:T nonS		232 A:C misS		
H1	124 C:T nonS		346 fs+1			
H2	815 fs−1	214 C:T nonS				
H3	94[‖] fs−2		124[‖] C:T nonS		nt	nt
H4						
H5					nt	nt
H6	827 fs+1	214 C:T nonS		40 G:A misS		

Table S2. Cont.

Evolved coculture	*sat*	*aps*A	*aps*B	*dsr*C	1999*	0916[†]
H7	26 fs+1	364 G:T nonS				
H8			175 C:T nonS	11 T:C misS		
H9					nt	nt
H10			118 fs−1	301 C:T misS		

*Gene DVU 1999 is annotated as a sulfate permease.
[†]Gene DVU 0916 is a regulator.
[‡]The base pair location of the mutation within the gene.
[§]The nucleotide change is specified in the case of single-nucleotide polymorphisms.
[¶]The nature of the mutation is indicated: fs, frameshift; + or −1 or more indicates the number; misS, missense; nonS, nonsense; syn, synonymous.
[#]Populations tested only by PCR and Sanger sequencing are underlined.
[||]The likely presence of multiple genotypes was evident in the chromatogram.
**Not tested by PCR and Sanger sequencing.

Table S3. Primers used in this study

Primer name	Primer sequence*	Application
Primers used to verify SR-negative mutations with Sanger sequencing		
DVU_2776_F	TTCATGACATTGTGGCATGTT	Amplification of *dsr*C to verify mutations
DVU_2776_R	GCGATTTGTTCTGCTGATGA	Amplification of *dsr*C to verify mutations
DVU_0847_F	ACGGACGGTAGATGAGCTTC	Amplification of *aps*A to verify mutations
DVU_0847_R	CACCACTCCTGAAGGTTCCA	Amplification of *aps*A to verify mutations
DVU_1295_F	GCCCTGCGAATACAGGTTAT	Amplification of *sat* to verify mutations
DVU_1295_R	CGTAGAAGTCGCCCACACC	Amplification of *sat* to verify mutations
DVU_0846_F	ACCGAAAGGTGGTGAATCC	Amplification of *aps*B to verify mutations
DVU_0846_R	CACCAAGTTGCGTTGTTGTC	Amplification of *aps*B to verify mutations
Primers used to construct deletion mutants		
SpecRpUC-F	CCAGCCAGGACAGAAATGCCTCG	Amplification of pUC-Sp^r fragment
SpecRpUC-R	ATGTGAGCAAAAGGCCAGCAAAAGGC	Amplification of pUC-Sp^r fragment
Kan gene Prom Nterm	CCGGAATTGCCAGCTGGGGCGC	Amplification of npt and upp fragment
upp gene Cterm	CTTACTTGGTGCCGAATATCTTGTCGC	Amplification of npt and upp fragment
DVU1295-upF	GCCTTTTGCTGGCCTTTTGCTCACAT ACCGTCGTACATGAGTCGGTTGATG	Amplification of upstream region of *sat*
DVU1295-upR	GCGACAAGATATTCGGCACCAAGTAAG ACCTTACATCCTCCAGATGCGTGATG	Amplification of upstream region of *sat*, specific for marker-exchange plasmid
DVU1295-dnF	GCGCCCCAGCTGGCAATTCCGG AAATACGAAGGAGGCACCGCAA	Amplification of downstream region of *sat*, specific for marker-exchange plasmid
DVU1295-dnR	CGAGGCATTTCTGTCCTGGCTGG TGCCCACATGCCATAGCGA	Amplification of downstream region of *sat*
DVU1295-MLD-upR	TTGCGGTGCCTCCTTCGTATTT ACCTTACATCCTCCAGATGCGTGATG	Amplification of upstream region of *sat*, specific for markerless deletion plasmid
DVU1295-MLD-dnF	CATCACGCATCTGGAGGATGTAAGGT AAATACGAAGGAGGCACCGCAA	Amplification of downstream region of *sat*, specific for markerless deletion plasmid
DVU0846-7-upF	GCCTTTTGCTGGCCTTTTGCTCACAT CGCAAGAGAAAAGGAACAGGCAATGTC	Amplification of upstream region of *apsBA*
DVU0846-7-upR	GCGACAAGATATTCGGCACCAAGTAAG ACCTTATCCTCCAACTCAAACAGAATTAAGGGT	Amplification of upstream region of *apsBA*, specific for marker-exchange plasmid
DVU0846-7-dnF	GCGCCCCAGCTGGCAATTCCGG GATGAGCACCAGGGCGGTT	Amplification of downstream region of *apsBA*, specific for marker-exchange plasmid
DVU0846-7-dnR	CGAGGCATTTCTGTCCTGGCTGG TGTGGCGAGCACGATGGAC	Amplification of downstream region of *apsBA*
DVU0846-7-MLD-upR	AACCGCCCTGGTGCTCATC ACCTTATCCTCCAACTCAAACAGAATTAAGGGT	Amplification of upstream region of *apsBA*, specific for markerless deletion plasmid
DVU0846-7-MLD-dnF	ACCCTTAATTCTGTTTGAGTTGGAGGATAAGGT GATGAGCACCAGGGCGGTT	Amplification of downstream region of *apsBA*, specific for markerless deletion plasmid
DVU0848-50-upF	GCCTTTTGCTGGCCTTTTGCTCACAT TCAGGCCAACCTCTGGGC	Amplification of upstream region of *qmoABC*
DVU0848-50-upR	GCGACAAGATATTCGGCACCAAGTAAG CCTTGGTATCCTCCCTACGTGTTTTGG	Amplification of upstream region of *qmoABC*, specific for marker-exchange plasmid
DVU0848-50-dnF	GCGCCCCAGCTGGCAATTCCGG CAGAACACCGGTCGGCG	Amplification of downstream region of *qmoABC*, specific for marker-exchange plasmid
DVU0848-50-dnR	CGAGGCATTTCTGTCCTGGCTGG CAGCTGCTGCACCTGCAG	Amplification of downstream region of *qmoABC*
DVU0848-50-MLD-upR	CGCCGACCGGTGTTCTG CCTTGGTATCCTCCCTACGTGTTTTGG	Amplification of upstream region of *qmoABC*, specific for markerless deletion plasmid
DVU0848-50-MLD-dnF	CCAAAACACGTAGGGAGGATACCAAGG CAGAACACCGGTCGGCG	Amplification of downstream region of *qmoABC*, specific for markerless deletion plasmid
SpecRpUC-up	GGGAAACGCCTGGTATCTTTATAGTCCT	Sequencing of all plasmid constructs
pMO719-XbaI-dn	TGGGTTCGTGCCTTCATCCG	Sequencing of all plasmid constructs
Km_int_Fwd_revcomp	CTCATCCTGTCTCTTGATCAGATCT	Sequencing of markerless deletion plasmid constructs
upp gene Cterm Out	GCTGAAGCGCATCGTGGACAA	Sequencing of markerless deletion plasmid constructs

*Underlined regions correspond to overhangs used for SLIC assembly of PCR products.

Table S4. ANOVAs testing the effects of several *D. vulgaris* sulfate reduction genotypes on coculture growth with three methanogens

Source of variation	df	SS	MS	*F*	*P*
Full model: growth rate					
D. vulgaris strain	6	0.011	0.0018	77.33	<0.0001
M. maripaludis strain	2	0.026	0.0129	548.1	<0.0001
D. vulgaris × *M. maripaludis*	12	0.012	0.0010	43.19	<0.0001
Error	42	0.001	0.0000		
Full model: yield					
D. vulgaris strain	6	0.063	0.010	84.88	<0.0001
M. maripaludis strain	2	0.323	0.161	1,312.8	<0.0001
D. vulgaris × *M. maripaludis*	12	0.151	0.013	102.4	<0.0001
Error	42	0.005	0.000		
Mutant only model: growth rate					
D. vulgaris strain	3	0.0003	0.0001	8.87	0.0004
M. maripaludis strain	2	0.0296	0.0148	1,174.31	<0.0001
D. vulgaris × *M. maripaludis*	6	0.0001	0.0000	1.53	0.2099
Error	24	0.0003	0.0000		
Mutant only model: yield					
D. vulgaris strain	3	0.0006	0.0002	2.03	0.1372
M. maripaludis strain	2	0.3850	0.1925	1,960.4	<0.0001
D. vulgaris × *M. maripaludis*	6	0.0022	0.0004	3.65	0.0102
Error	24	0.0024	0.0000		

D. vulgaris strains include D+, D−, DA, and (excluded from mutant-only model) JW710, and the *aps*AB, *sat*, and *qmo*ABCD mutants (see Fig. 3, *SI Methods*, and *Results* for detailed descriptions of strains). *M. maripaludis* tests the effect of M+, M−, or MA on coculture growth, and *D. vulgaris* × *M. maripaludis* tests whether these effects depend on which *D. vulgaris* it is paired with.

Table S5. ANOVAs testing the effects on coculture growth of genetic variation among EPD lines from cocultures U9, H6, and H2

Source of variation	df	SS	MS	*F*	*P*
Growth rate					
Evolved coculture	2	0.0008	0.0004	8.75	0.0011
EPD lines (coculture)	11	0.0092	0.0008	18.47	<0.0001
Error	28	0.0013	0.00005		
Contrasts: growth rate					
U9: 2 SR+ = 2 SR− (0 1 1 −1 −1)	1	0.0030	0.0030	66.34	<0.0001
H6: 2SR+ = 2SR− (0 1 1 −1 −1)	1	0.0036	0.0036	78.64	<0.0001
H2: 2*SR+ = 2SR− (0 2 −1 −1)	1	0.0004	0.0004	9.82	0.0040
Yield					
Evolved coculture	2	0.0404	0.0202	123.7	<0.0001
EPD lines (coculture)	11	0.0188	0.0017	10.5	<0.0001
Error	28	0.0046	0.0002		
Contrasts: yield					
U9: 2 SR+ = 2 SR− (0 1 1 −1 −1)	1	0.0014	0.0014	8.62	0.0066
H6: 2SR+ = 2SR− (0 1 1 −1 −1)	1	0.0117	0.0117	71.8	<0.0001
H2: 2*SR+ = 2SR− (0 2 −1 −1)	1	0.00003	0.00003	0.21	0.6483

Evolved coculture tests whether EPD cultures differ between U9, H6, or H2. EPD lines (coculture) tests variation among EPD lines and is nested within evolved coculture. SR+ and SR− refer to EPD cultures that are SR-positive or SR-negative and the preceding integer specifies the number of EPD cultures tested for the indicated coculture lineage. The coefficients per EPD in each contrast are listed.

Table S6. Strains and plasmids used in this study

Strain or plasmid	Genotype and relevant features	Source
Desulfovibrio vulgaris strains		
Desulfovibrio vulgaris Hildenborough	Wild-type strain, ATCC 29579	ATCC
JW710	*Desulfovibrio vulgaris* Hildenborough Δ*upp* $5FU^r$	Ref. 1
JW9257	JW710 Δ*apsB aph(3′)-II*:*upp* $G418^r$ $5FU^s$	This study
JW9259	JW710 Δ*apsBA*	This study
JW9261	JW710 Δ*qmoABC aph(3′)-II*:*upp* $G418^r$ $5FU^s$	This study
JW9263	JW710 Δ*qmoABC* $5FU^r$	This study
JW9269	JW710 Δ*sat aph(3′)-II*:*upp* $G418^r$ $5FU^s$	This study
JW9271	JW710 Δ*sat* $5FU^r$	This study
Escherichia coli strains		
α-select	*deoR endA1 recA1 relA1 gyrA96 hsdr17*(r_k^- m_k^+) *supE44 thi-1* Δ(*lacZYA-argFU169*) φ80δ*lacZ*Δ*M15* F- λ-	Bioline
Plasmids		
pCR8/GW/TOPO	Plasmid used to amplify pUC-Sp^r fragment, Sp^r	Life Technologies
pMO746	Plasmid containing *aph(3′)-II:upp* 2-gene operon, Ap^r, Km^r	Ref. 2
pMO9256	Plasmid containing upstream and downstream regions of *apsBA* on either side of *aph(3′)-II:upp* (used to construct marker-exchange deletion), Sp^r, Km^r	This study
pMO9258	Plasmid containing upstream and downstream regions of *apsBA* (used to construct markerless deletion), Sp^r	This study
pMO9260	Plasmid containing upstream and downstream regions of *qmoABC* on either side of *aph(3′)-II:upp* (used to construct marker-exchange deletion), Sp^r, Km^r	This study
pMO9262	Plasmid containing upstream and downstream regions of *qmoABC* (used to construct markerless deletion), Sp^r	This study
pMO9268	Plasmid containing upstream and downstream regions of *sat* on either side of *aph(3′)-II:upp* (used to construct marker-exchange deletion), Sp^r, Km^r	This study
pMO9270	Plasmid containing upstream and downstream regions of *sat* (used to construct markerless deletion), Sp^r	This study

1. Keller KL, Bender KS, Wall JD (2009) Development of a markerless genetic exchange system for *Desulfovibrio vulgaris* Hildenborough and its use in generating a strain with increased transformation efficiency. *Appl Environ Microbiol* 75(24):7682–7691.
2. Parks JM, et al. (2013) The genetic basis for bacterial mercury methylation. *Science* 339(6125):1332–1335.