Supporting Information

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SI Materials and Methods

General Procedures. All chemicals were purchased from Sigma-Aldrich, except for d_9-trimethylamine (TMA) hydrochloride, (CDN Isotopes) and (trimethyl-d_9)-choline chloride (Cambridge Isotope Laboratories). Methanol and water used for liquid chromatography-mass spectrometry (LC-MS) were B&J Brand high-purity solvents (Honeywell Burdick & Jackson).

Oligonucleotide primers were synthesized by Integrated DNA Technologies. Recombinant plasmid DNA was purified with a Qiaprep kit from Qiagen. Gel extraction of DNA fragments and restriction endonuclease cleanup were performed using an Illustra GFX PCR DNA and Gel Band Purification kit from GE Healthcare. DNA sequencing was performed at the GENEWIZ facility, Boston. SDS/PAGE gels were purchased from Bio-Rad.

Optical densities of *Desulfovibrio desulfuricans*, *Desulfovibrio alaskensis*, and *Escherichia coli* cultures were determined with a GENESYS 20 spectrophotometer (Thermo Scientific) by measuring absorbance at 600 nm.

LC-MS analysis was performed on an Agilent 6410 Triple Quadrupole LC/MS instrument (Agilent Technologies), operated in multiple reaction monitoring (MRM) mode. The LC analysis was performed in positive ion mode, using a Bio-Bond (Dikma Technologies) C4 column (5 μm, 4.6 mm × 50 mm), preceded by a C4 precolumn (3.5 μm, 2.0 mm × 20 mm). The mobile phase consisted of a 50/50 water/methanol mixture, supplemented with 5 mM ammonium formate and 0.1% formic acid as solvent modifiers. The flow rate was maintained at 0.3 mL/min for each run. Run time per sample was 6 min, and the first 1.8 min of flow through was sent to waste. The injection volume was 3 μL. Blanks consisting of 50/50/0.025 (vol/vol/vol) acetonitrile/water/formic acid were run in between each sample. Samples and blanks were introduced via an electrospray ionization (ESI) source. The capillary voltage was set to 4.0 kV, the fragmentor voltage to 110 V, and the skimmer voltage to 15 V. The drying gas temperature was maintained at 300 °C with a flow rate of 10 L/min and a nebulizer pressure of 45 psi. The precursor-product ion pairs used in MRM mode for d_9-TMA were: m/z 155.1→ m/z 127.1 [collision energy, (CE = 14 V), m/z 155.1→ m/z 66.2 (CE = 43 V); and for TMA they were: m/z 146.1→ m/z 118.1 (CE = 14 V), m/z 146.1→ m/z 58.1 (CE = 43 V)]. MS1 resolution was set to wide, MS2 resolution was set to unit, the time filter width used was 0.07 min, and the ΔEMV (Electron Multiplier Voltage) was 400 V. Data analysis was performed with Mass Hunter Workstation Data Acquisition software (Agilent Technologies). Peak intensities from the transitions indicated in each section, at a retention time of ∼2.4 min, were used for the final quantification of TMA or d_9-TMA. To assess the statistical significance of individual data points, a Student t test (two-tailed, heteroscedastic) was used to calculate a P value testing the null hypothesis that the amount of d_9-TMA is the same in the samples indicated on each graph.

Perpendicular mode X-band electron paramagnetic resonance (EPR) spectra were recorded on a Bruker ElexSysE500 EPR instrument with a 100 K–600 K Digital Temperature Control system, ER 4131VT (Bruker BioSpin), and data acquisition and baseline corrections were performed with Xepr software (Bruker). The magnetic field was calibrated with a standard sample of α,γ-bisdiphenylene-β-phenylallyl (BDPA), g = 2.0026; Bruker).The experimental spectra were modeled with EasySpin for Matlab to obtain g values, hyperfine coupling constants, and line widths (1). EPR spectra represent the average of nine scans and were recorded under nonsaturating conditions: temperature, 120 K; center field, 3,360 Gauss; range, 150 Gauss; microwave power, 63.25 μW; microwave frequency, 9.433602 GHz (choline fermentation media), 9.436572 GHz (pyruvate fermentation media); modulation amplitude, 0.1 mT; modulation frequency, 100 kHz; time constant, 40.96 ms; conversion time, 163.84 ms; and scan time, 167.77 s.

Growth of *D. desulfuricans* ATCC 27774 on Various Carbon Sources and Quantitation of TMA Production from Choline. *D. desulfuricans* ATCC 27774 was obtained from João Nuno Carita (Universidade Nova de Lisboa, Lisbon). Lactate-sulfate (LS) medium was used for routine cultivation and was prepared as previously described (2). All concentration values indicated correspond to final values. LS medium contains sodium lactate (60 mM, 1.2% wt/vol), sodium sulfate (50 mM), magnesium sulfate (8 mM), ammonium chloride (5 mM), HEPES pH 7.2 (25 mM), calcium chloride (0.6 mM), yeast extract (Becton, Dickinson and Company) (0.1%), trace mineral solution [nitrilotriacetic acid trisodium salt (0.62 mM), iron (II) sulfate heptahydrate (19 μM), manganese (II) chloride tetrahydrate (6.3 μM), cobalt (II) chloride hexahydrate (8.9 μM), copper (II) chloride dihydrate (1.5 μM), zinc (II) sulfate heptahydrate (9.1 μM), sodium molybdate dihydrate (0.5 μM), and sodium selenate (1.0 μM)], vitamin solution (3) [pyridoxine hydrochloride (96 nM), thiamine hydrochloride (30 nM), riboflavin (27 nM), calcium pantothenate (42 nM), lipoic acid (48 nM), *p*-aminobenzoic acid (73 nM), nicotinic acid (81 nM), vitamin B_{12} (7 nM), biotin (16 nM), folic acid (9 nM), and mercaptoethanesulfonic acid (141 nM)], and resazurin (2.5 μM). The pH of the medium was adjusted to 7.2. The medium was autoclaved for 5 min, sparged for 30 min with argon, dispensed into 18 × 150 mm modified Hungate tubes (Chemglass Life Sciences; CLS-4209-10) and capped with butyl rubber stoppers and aluminum seals (10 mL media per tube). The tubes were autoclaved for an additional 20 min. After cooling, anaerobic and sterile aqueous solutions of potassium phosphate dibasic (2.2 mM), sodium bicarbonate (8 mM), and cysteine-HCl (0.04%) were added. Tubes of anaerobic media were stored under a headspace of argon.

Lactate fermentation media (L) was prepared in a similar manner to the LS media except that sodium sulfate was replaced with sodium chloride (100 mM) and magnesium sulfate was replaced with magnesium chloride (8 mM). Choline-sulfate (CS) and choline fermentation (C) media were prepared in a similar manner to the LS and L media, except that it was supplemented with 0.01 mg/mL iron (III) ammonium citrate, and sodium lactate was replaced by choline chloride (60 mM), which was added as an anaerobic, filter-sterilized aqueous solution shortly before inoculation. Ethanolamine- and puruvate-containing media [ethanolamine-sulfate (ES), ethanolamine fermentation (E), pyruvate-sulfate (PS), and pyruvate fermentation (P)] were prepared like CS and C media, except that choline was replaced with ethanolamine hydrochloride (60 mM) or sodium pyruvate (60 mM).

For each media type, two 18 × 150 mm modified Hungate tubes containing 10 mL of medium and N_2 in the headspace were inoculated with 0.1 mL of an overnight LS starter culture (OD_{600} = 0.85) and incubated at 37 °C. OD_{600} was measured periodically until the cultures reached stationary phase. Growth was observed on LS, CS, C, PS, and P media (Fig. S1*A*).

To confirm and quantify TMA formation during growth on choline, *D. desulfuricans* ATCC 27774 was grown on CS and C media containing (trimethyl-d_9)-choline (60 mM). This growth substrate was added as an anaerobic, filter-sterilized aqueous

solution shortly before inoculation. Four 10 mL replicates for each media type (CS, and C) were inoculated with 0.1 mL of an overnight LS starter culture (OD_{600} = 0.85) and grown at 37 °C until reaching stationary phase (2–3 d). The concentration of d_9-TMA in culture media (Fig. S1*B*) was determined using LC-MS after derivatization with ethyl bromoacetate using a published procedure (4). Briefly, to a 10-mL glass vial sealed with a 13 × 20 mm sleeve stopper (VWR) was added 100 μL of bacterial culture filtered through a 13-mm, 0.22-μm pore-size Acrodisc syringe filter with HT Tuffryn Membrane (Pall Life Sciences), 100 μL of TMA (43.3 mM in water), 10 μL of concentrated ammonia (7 M in methanol), and 120 μL of ethyl bromoacetate (20 mg/mL in acetonitrile). This mixture was incubated at room temperature for 30 min and then quenched by adding 4 mL of infusion solution [acetonitrile/water/formic acid, 50/50/0.025 (vol/vol/vol)]. A 0.5-μL aliquot of the resulting mixture was further diluted 2,000-fold with infusion solution and analyzed by LC-MS (3 μL injection volume) using the parameters described in *SI Materials and Methods*, *General Procedures*. The transitions used for quantitation were *m/z* 155.1→ 127.1 for d_9-TMA and *m/z* 146.1→ 118.1 for TMA. Media blanks consisted of 10 mL of uninoculated CS and C media containing (trimethyl-d_9)-choline (60 mM). Four tubes per type of media were incubated at 37 °C alongside the bacterial cultures until the cultures reached stationary phase.

Cloning CutC (Dde_3282) and CutD (Dde_3281) from *D. alaskensis* G20. Genomic DNA from *D. alaskensis* G20 was isolated from a 10-mL LS culture using the UltraClean Microbial DNA Isolation kit from MoBio Laboratories. The sequences of primers used for amplification of *cutC* (Dde_3282) and *cutD* (Dde_3281) genes are provided in Table S5. Primers were designed to amplify both of the individual genes as well as the 3529-bp region (3270700–3274229) encoding both CutC and CutD. CutC was PCR amplified from genomic DNA using forward primer Dde-3282-NdeI-start and reverse primer Dde-3282-XhoI-nostop. All PCR reactions (set up in triplicate) contained 25 μL of PfuTurbo Hotstart master mix (Stratagene), 2 μL of genomic DNA template, and 100 pmol of each primer in a total volume of 50 μL. Thermocycling was carried out in a C1000 Gradient Cycler (Bio-Rad) using the following parameters: denaturation for 1 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 1 min at 68.4 °C, 5 min at 70 °C, and a final extension time of 10 min at 70 °C. PCR reactions were analyzed by agarose gel electrophoresis with ethidium bromide staining, pooled, and purified. Identical conditions were used to amplify CutD (forward primer Dde-3281-NdeI-start + reverse primer Dde-3281-XhoI-stop) as well as CutC + CutD (forward primer Dde-3282-NdeI-start + reverse primer Dde-3281-XhoI-stop).

Amplified fragments were digested with NdeI and XhoI (New England Biolabs, NEB) for 2.5 h at 37 °C. Digests contained 1 μL of water, 3 μL of NEB buffer 4 (10×), 3 μL of BSA (10×), 20 μL of PCR product, 1.5 μL of NdeI (20 units/μL), and 1.5 μL of XhoI (20 units/μL). Restriction digests were purified directly using agarose gel electrophoresis and the gel fragments were further purified using the Illustra GFX kit. The digests were ligated into linearized expression vectors using T4 DNA ligase (NEB). The CutC + CutD and CutD digests were ligated into linearized pET-29b(+) to encode untagged and C-terminal His_6-tagged constructs, respectively, whereas the CutC digest was ligated into linearized pET-28a(+) to encode an N-terminal His_6-tagged construct. Ligations were incubated at 16 °C for 24 h and contained 3.5 μL water, 1 μL T4 ligase buffer (10×), 0.5 μL digested vector, 3 μL digested insert DNA, and 2 μL T4 DNA ligase (400 units/μL). A total of 5 μL of each ligation was used to transform a single tube of chemically competent *E. coli* TOP10 cells (Invitrogen). The identities of the resulting pET-29b-CutC/CutD, pET-29b-CutD, and pET-28a-CutC constructs were confirmed by sequencing purified plasmid DNA. These constructs were transformed into chemically competent *E. coli* BL21 (DE3) cells (Invitrogen) and stored at −80 °C as frozen LB/glycerol stocks.

Construction of CutC (Dde_3282) C489A and G821A Mutants. The C489A and G821A mutants of CutC (Dde_3282) were constructed separately in pET-29b-CutC/CutD using site-directed mutagenesis. The CutC (Ddes_1357) homology model identified these amino acid positions (which correspond to C491 and G823 in Ddes_1357 and are conserved in all glycyl radical enzymes) as sites of the thiyl and glycyl radicals involved in catalysis (5). The sequences of primers used for mutagenesis are found in Table S5. The C489A mutant was constructed via PCR amplification of pET-29b-CutC/CutD using forward primer Dde3282-QC-C489A-for and reverse primer Dde3282-QC-C489A-rev. The G821A mutant was constructed via PCR amplification of pET-29b-CutC/CutD using forward primer Dde3282-QC-G821A-for and reverse primer Dde3282-QC-G821A-rev.

PCR reactions contained 36 μL of PfuTurbo DNA polymerase (2.5 units/μL; Stratagene), 5 μL of Pfu DNA polymerase reaction buffer (10×), 1 μL of 10 mM dNTP Mix (Bio-Rad), 50 ng DNA template, and 125 ng of each forward and reverse primer in a total volume of 50 μL. Thermocycling was carried out using the following parameters: denaturation for 30 s at 95 °C, followed by 18 cycles of 30 s at 95 °C, 5 min at 55 °C, and 9 min at 68 °C. Upon cooling to room temperature, 1 μL of DpnI (NEB) was added to each tube, and the resulting mixtures were incubated at 37 °C. After 1 h, an additional 1 μL of DpnI was added to each tube and incubation continued for an additional hour at 37 °C. A total of 5 μL of each digestion was used to transform a single tube of chemically competent *E. coli* TOP10 cells. The identity of the resulting pET-29b-CutC/CutD-C489A or -G821A constructs was confirmed by sequencing purified plasmid DNA. Chemically competent *E. coli* BL21(DE3) cells were transformed with pure pET-29b-CutC/CutD-C489A or -G821A plasmid DNA and stored at –80 °C as frozen LB/glycerol stocks.

1. Stoll S, Schweiger A (2006) EasySpin, a comprehensive software package for spectral simulation and analysis in EPR. *J Magn Reson* 178(1):42–55.
2. Krumholz LR, Bryant MP (1986) *Eubacterium oxidoreducens* sp. nov. requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol, and quencetin. *Arch Microbiol* 144:8–14.
3. Steger JL, Vincent C, Ballard JD, Krumholz LR (2002) *Desulfovibrio* sp. genes involved in the respiration of sulfate during metabolism of hydrogen and lactate. *Appl Environ Microbiol* 68(4):1932–1937.
4. Johnson DW (2008) A flow injection electrospray ionization tandem mass spectrometric method for the simultaneous measurement of trimethylamine and trimethylamine *N*-oxide in urine. *J Mass Spectrom* 43(4):495–499.
5. Lehtiö L, Goldman A (2004) The pyruvate formate lyase family: Sequences, structures and activation. *Protein Eng Des Sel* 17(6):545–552.

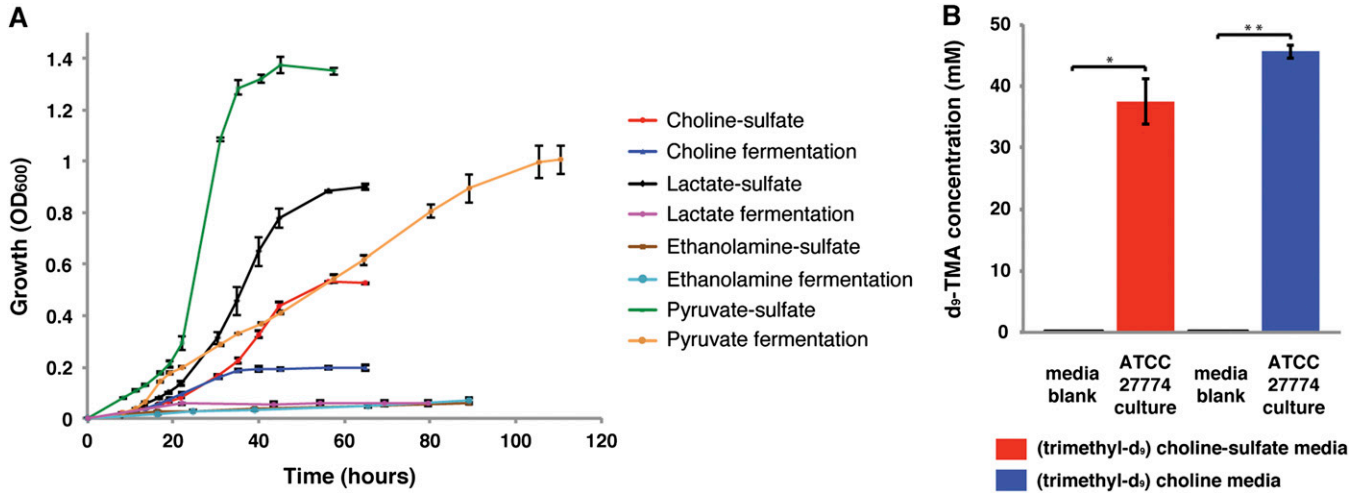

Fig. S1. Choline utilization by *D. desulfuricans* ATCC 27774 proceeds via formation of trimethylamine (TMA). (*A*) Growth of *D. desulfuricans* ATCC 27774 using various carbon sources. The data shown are the average OD_{600} values of two cultures. Error bars represent SD. (*B*) LC-MS quantification of d_9-TMA production during *D. desulfuricans* ATCC 27774 growth on (trimethyl-d_9)-choline. Choline-sulfate and choline fermentation media contained 60 mM (trimethyl-d_9)-choline chloride. Bar graphs represent the mean ± SEM of four replicates. $*P < 0.002$; $**P < 10^{-4}$.

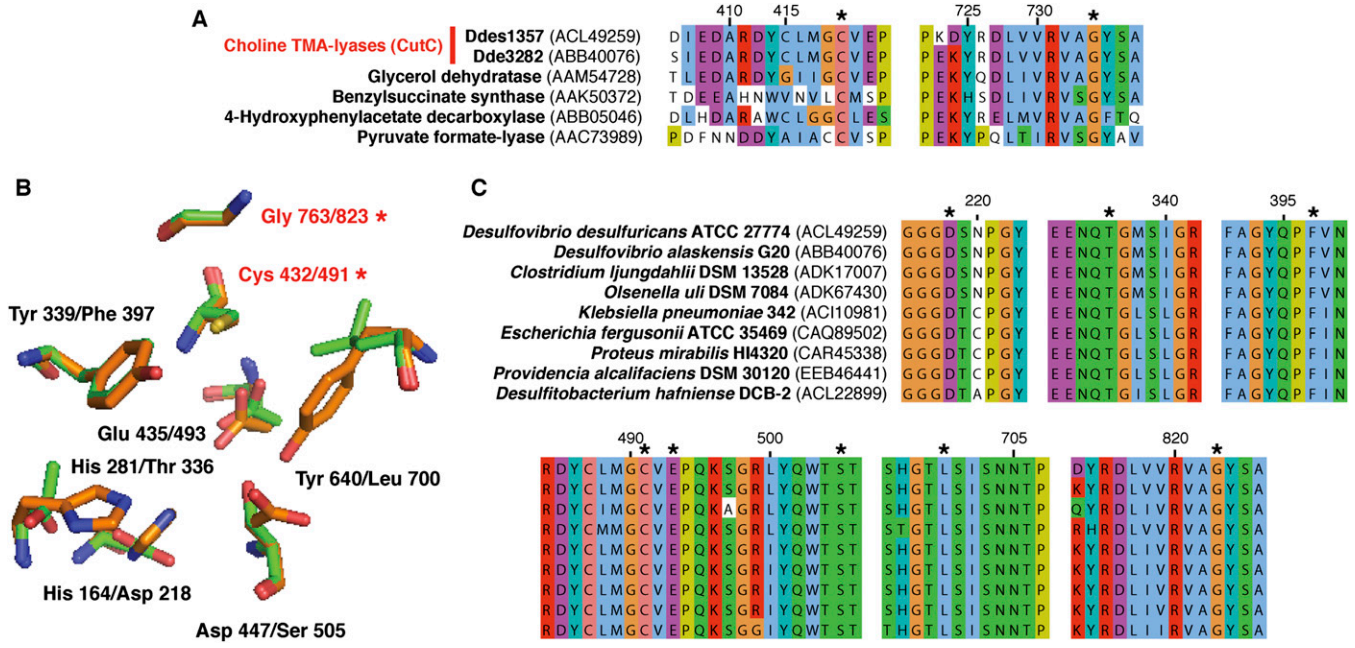

Fig. S2. Bioinformatic analyses of choline TMA-lyase CutC. (*A*) Multiple sequence alignment of putative choline TMA-lyases Ddes_1357 and Dde_3282 with characterized glycyl radical enzymes reveals conservation of catalytic active site residues. Asterisks indicate conserved active site cysteine and glycine residues involved in radical catalysis. *E. coli* pyruvate formate-lyase numbering is shown. GenBank accession no. identifies amino acid sequences used. (*B*) Overlay of the active site residues of B_{12}-independent glycerol dehydratase and choline TMA-lyase. The crystal structure of glycerol dehydratase from *Clostridium butyricum* is shown in orange and the homology model of CutC (Ddes1357) from *D. desulfuricans* is shown in green. Asterisks indicate conserved active site residues involved in radical generation. (*C*) Multiple sequence alignment of putative choline TMA-lyases from different bacterial genera shows conservation of active site residues. Asterisks indicate conserved active site residues identified from the homology model shown in Fig. S2*B*. *D. desulfuricans* ATCC 27774 CutC (Ddes_1357) numbering is shown. GenBank accession no. identifies amino acid sequences used.

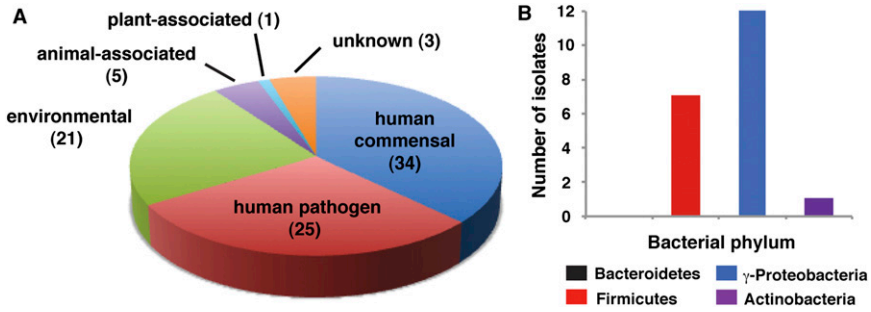

Fig. S3. Environmental and phylogenetic distribution of choline utilization. (*A*) Sources of sequenced bacterial isolates that possess a complete *cut* gene cluster. (*B*) Phylogenetic distribution of putative choline-degrading human gastrointestinal tract isolates.

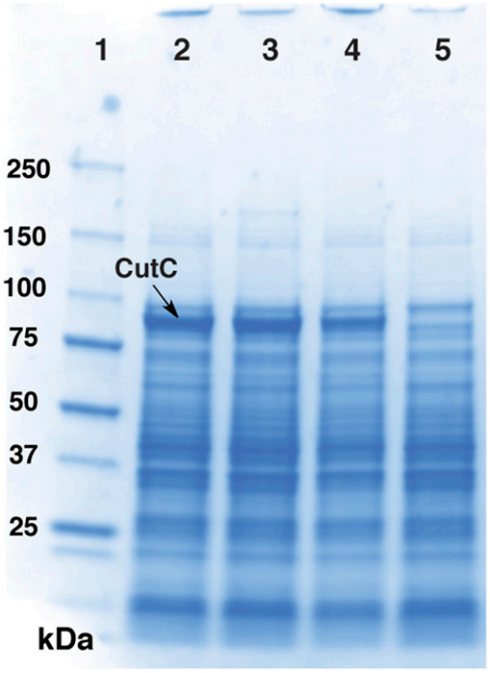

Fig. S4. SDS-PAGE of cell lysates from heterologous expressions of CutC and CutD in *E. coli* using a 4–15% (wt/vol) polyacrylamide Tris-HCl gel. Lane 1, 10–250 kDa protein ladder (New England Biolabs); lane 2, wild-type CutC + CutD; lane 3, CutC C498A mutant + CutD; lane 4, CutC G821A mutant + CutD; and lane 5, empty pET-29b vector control. The calculated molecular weight of CutC (Dde_3282) is 96 kDa.

Table S1. Annotation of choline utilization (*cut*) gene cluster in *Desulfovibrio desulfuricans* ATCC 27774

Gene	GenBank accession no. (protein sequence)	Size (bp/aa)	Homolog in Eut operon (% amino acid ID/similarity)	Putative function
cutA Ddes_1355	ACL49257	597/188	EutM (51/73) EutK (37/62)	Microcompartment structural protein
cutB Ddes_1356	ACL49258	1475/492	EutE (29/48)	CoA acylating aldehyde dehydrogenase
cutC Ddes_1357	ACL49259	2574/848	None	Glycyl radical enzyme
cutD Ddes_1358	ACL49260	933/310	None	Glycyl radical enzyme activating protein
cutE Ddes_1359	ACL49261	609/202	EutM (50/67) EutK (48/68)	Microcompartment structural protein
cutF Ddes_1360	ACL49262	1578/525	EutE (32/50)	CoA acylating aldehyde dehydrogenase
cutG Ddes_1361	ACL49263	288/99	EutM (71/83) EutK (47/71)	Microcompartment structural protein
cutH Ddes_1362	ACL49264	684/227	None	Propanediol utilization protein
cutI Ddes_1363	ACL49265	822/273	EutJ (43/62)	Putative chaperonin
cutJ Ddes_1364	ACL49266	639/212	None	Propanediol utilization protein
cutK Ddes_1365	ACL49267	288/99	EutN (38/61)	Microcompartment structural protein
cutL Ddes_1366	ACL49268	549/182	EutM (40/57) EutK (41/60)	Microcompartment structural protein
cutM Ddes_1367	ACL49269	336/111	None	Propanediol utilization protein
cutN Ddes_1368	ACL49270	285/94	EutM (71/84)	Microcompartment structural protein
cutO Ddes_1369	ACL49271	1107/368	EutG (36/51)	Alcohol dehydrogenase
cutP Ddes_1370	ACL49272	1326/441	None	NADH dehydrogenase
cutQ Ddes_1371	ACL49273	552/183	EutM (35/58)	Microcompartment structural protein
cutR Ddes_1372	ACL49274	348/115	EutS (52/72) EutM (34/57)	Microcompartment structural protein
cutS Ddes_1373	ACL49275	435/144	EutP (34/52)	Ethanolamine utilization protein

Table S2. Putative *cut* gene clusters in sequenced bacterial genomes. Human Microbiome Project (HMP) reference strains are indicated with an asterisk (*)

Organism	Gene encoding choline TMA-lyase	GenBank accession no. (protein sequence)	Homology to Ddes_1357 (% amino acid ID/ similarity)	Source of isolate
Desulfovibrio desulfuricans ATCC 27774	Ddes_1357	ACL49259	100/100	Rumen of a sheep
Desulfovibrio alaskensis G20	Dde_3282	ABB40076	83/91	Oil well corrosion site
Clostridium hathewayi DSM 13479*	CLOSTHATH_02755	EFC99034	81/91	Human feces collected in UK
Clostridium phytofermentans ISDg	Cphy_1417	ABX41792	78/89	Forest soil near the Quabbin Reservoir in Massachusetts
Clostridium saccharolyticum WM1	Closa_4018	ADL06529	78/90	Sewage sludge in Canada
Desulfovibrio salexigens DSM 2638	Dsal_0468	ACS78535	79/88	Mud in British Guyana
Clostridium ljungdahlii DSM 13528	PflB3	ADK17007	78/90	Chicken yard waste
Clostridium carboxidivorans P7	CLCAR_0126	EFG89933	78/90	Agricultural settling lagoon at Oklahoma State University
Clostridium citroniae WAL-17108*	HMPREF9469_00639	EHF00461	77/89	Human feces collected in Canada
Clostridium botulinum Ba4 str. 657	CLJ_B2322	ACQ52082	78/90	Infant botulism case in Texas in 1976
Clostridium botulinum Bf	CBB_2390	EDT85049	78/90	Infant botulism case
Clostridium sporogenes PA3679	IYC_13304	EHN14588	78/90	Spoiled canned corn, nonpathogenic
Clostridium botulinum A2 str. Kyoto	CLM_2326	ACO85636	78/90	Infant botulism in Kyoto, Japan in 1978
Clostridium botulinum A str. ATCC 3502	CBO2118	CAL83658	78/89	Botulism case
Clostridium botulinum A str. ATCC 19397	CLB_2055	ABS33990	78/89	Laboratory strain probably from food-borne botulism cases in the western US
Clostridium botulinum BoNT/ A1 Hall	CLC_2060	ABS38084	78/89	Harvard University, 1947
Clostridium botulinum F str. Langeland	CLI_2161	ABS41623	78/89	Home-prepared liver paste involved in an outbreak of food-borne botulism on the island of Langeland in Denmark in 1958
Clostridium botulinum NCTC 2916	CBN_2167	EDT81181	78/89	Botulism case
Clostridium botulinum F str. 230613	CBF_2145	ADF99816	78/89	Isolated in China
Clostridium botulinum H04402 065	H04402_02123	CBZ03931	78/89	Botulism patient in UK in 2004
Clostridium botulinum B1 str. Okra	CLD_2523	ACA46788	78/89	Food-borne botulism incident in US
Clostridium botulinum D str. 1873	CLG_B0723	EES91121	78/89	Isolated from ham and was associated with the only known human outbreak of type D botulism, in Chad in 1958
Clostridium botulinum A3 str. Loch Maree	CLK_1571	ACA56033	77/89	Duck liver paste during a botulism outbreak at a hotel in the Scottish highlands in 1922
Clostridium botulinum BKT015925	CbC4_1734	AEB76410	77/89	Outbreak in a Swedish poultry farm
Desulfotalea psychrophila LSv54	DP1823	CAG36552	77/89	Marine sediments off the coast of Svalbard
Clostridium sporogenes ATCC 15579*	CLOSPO_02864	EDU36695	78/90	Human feces
Clostridium botulinum B str. Eklund 17B	CLL_A1453	ACD21782	77/89	Nonpathogen, marine sediments taken off the coast of Washington
Clostridium botulinum E1 str. 'BoNT E Beluga'	CLO_2328	EES47897	77/89	Fermented whale flippers, food-borne botulism outbreak in Canada
Clostridium tetani E88	CTC_01449	AAO36007	77/90	Pathogen
Clostridiales bacterium 1_7_47_FAA*	CBFG_00666	EEQ56956	76/87	Human feces

Table S2. Cont.

Organism	Gene encoding choline TMA-lyase	GenBank accession no. (protein sequence)	Homology to Ddes_1357 (% amino acid ID/ similarity)	Source of isolate
Clostridium botulinum E3 str. Alaska E43	CLH_1376	ACD51097	77/89	Salmon eggs associated with a food-borne case of botulism in Alaska
Alkaliphilus metalliredigens QYMF	Amet_3836	ABR49953	77/89	Borax leachate ponds
Alkaliphilus oremlandii OhILAs	Clos_2398	ABW19930	77/90	Anoxic sediments from the Ohio River, Pennsylvania
Anaerococcus hydrogenalis DSM 7454*	ANHYDRO_00917	EEB36265	75/87	Human feces
Anaerococcus hydrogenalis ACS-025-V-Sch4*	HMPREF9246_1434	EGC83561	75/87	Human vaginal cavity
Anaerococcus vaginalis ATCC 51170*	HMPREF0078_1374	EEU12078	75/87	Human vaginal discharge
Anaerococcus tetradius ATCC 35098*	HMPREF0077_1337	EEI82584	75/87	Human vaginal flora, associated with bacterial vaginosis (BV)
Collinsella tanakaei YIT 12063*	HMPREF9452_00749	EGX67047	74/87	Human feces
Olsenella uli DSM 7084*	Olsu_0306	ADK67430	72/87	Plaque from human gingival crevices
Olsenella sp. oral taxon 809 str. F0356*	HMPREF1008_01171	EHF01547	72/86	Oral cavity
Streptococcus dysgalactiae subsp. equisimilis GGS_118	complement (AB479807.1:24102.0.26648)	BAI63325	71/84	Clinical isolate, toxic shock syndrome
Streptococcus dysgalactiae subsp. equisimilis SK1249*	HMPREF9964_1417	EGL49759	71/84	Oral cavity
Streptococcus dysgalactiae subsp. equisimilis ATCC 12394	SDE12394_10005	ADX25417	71/84	Bovine udder infection
Paenibacillus dendritiformis C454	PDENDC454_17188	EHQ61018	71/84	—
Clostridium asparagiforme DSM 15981*	CLOSTASPAR_04587	EEG53372	83/93	Human feces
Desulfotomaculum reducens MI-1	Dred_3278	ABO51778	65/81	Heavy-metal–contaminated sediment collected at the Mare Island Naval Shipyard on San Francisco Bay
Desulfosporosinus meridiei DSM 13257	DesmerDRAFT_0460	EHC17003	63/80	Groundwater contaminated with aromatic compounds from motor fuel in sandy soil from Western Australia
Desulfotomaculum ruminis DSM 2154	Desru_2090	AEG60342	64/80	Rumen of hay-fed sheep
Desulfosporosinus youngiae DSM 17734	DesyoDRAFT_5132	EHQ92066	63/80	Constructed wetland sediment, South Carolina
Desulfosporosinus orientis DSM 765	Desor_5421	AET70797	63/80	Soil at pumping station near rising main Rangoon Road, Singapore
Desulfitobacterium hafniense DP7*	HMPREF0322_04110	EHL05181	64/79	Human feces
Desulfitobacterium dehalogenans ATCC 51507	DesdeDRAFT_0645	EHP61312	64/79	Sediment in freshwater pond, Athens, Georgia
Desulfitobacterium hafniense DCB-2	Dhaf_4905	ACL22899	64/79	Municipal sludge, Denmark
Desulfosporosinus sp. OT	DOT_2444	EGW39601	63/79	Sediment in the Norilsk hydrometallurgy tailings dam
Desulfitobacterium hafniense Y51	DSY5006	BAE86795	65/80	Soil contaminated with tetrachloroethene in Japan
Klebsiella oxytoca 10–5245*	HMPREF9689_00016	EHT04374	65/80	Human urine
Klebsiella sp. MS 92–3*	HMPREF9538_05494	EGF60105	62/78	Human feces
Klebsiella pneumoniae 342	KPK_4883	ACI10981	62/78	Interior of nitrogen-efficient maize plants
Klebsiella oxytoca 10–5250*	HMPREF9694_03855	EHT07921	65/80	Urogenital tract
Klebsiella variicola At-22	Kvar_4520	ADC60394	62/78	*Atta cephalotes* fungus garden

Table S2. Cont.

Organism	Gene encoding choline TMA-lyase	GenBank accession no. (protein sequence)	Homology to Ddes_1357 (% amino acid ID/ similarity)	Source of isolate
Klebsiella pneumoniae subsp. rhinoscleromatis ATCC 13884*	HMPREF0484_5138	EEW38822	62/78	Human airways sample, nose of patient in Sumatra
Enterobacter aerogenes KCTC 2190	EAE_10070	AEG96930	64/80	Human pathogen, airways
Klebsiella oxytoca 10–5242*	HMPREF9686_04809	EHS90956	64/80	Human bone
Klebsiella oxytoca 10–5243*	HMPREF9687_04958	EHS89002	64/79	Human blood
Klebsiella oxytoca KCTC 1686	KOX_09770	AEX03680	64/80	—
Escherichia fergusonii ECD227	ECD227_1338	EGC95100	64/79	Broiler chicken
Escherichia fergusonii B253	ERIG_01261	EGC08120	64/79	—
Klebsiella oxytoca 10–5246*	HMPREF9690_00446	EHT14116	65/80	Human blood
Vibrio furnissii NCTC 11218	vfu_A00327	ADT85554	62/78	Estuarine water, UK; causes gastroenteritis, zoonotic disease
Escherichia fergusonii ATCC 35469*	EFER_1995	CAQ89502	65/80	Human feces of 1-y-old boy
Aeromonas hydrophila subsp. hydrophila ATCC 7966	AHA_1330	ABK39643	65/81	Canned milk from US; food poisoning, gastroenteritis, septicemia
Escherichia coli O7:K1 str. CE10	CE10_4248	AEQ14949	65/80	Cerebrospinal fluid of a neonate with meningitis, US
Escherichia coli F11	EcF11_0690	EDV68614	65/80	20-y-old woman presenting her first case of cystitis with bacteriuria
Escherichia coli UM146	UM146_22045	ADN73733	65/80	Ileum of a patient with Crohn disease
Escherichia coli AA86	ECAA86_03971	EGH36471	65/80	Feces of healthy cow
Escherichia coli IAI39	ECIAI39_4211	CAR20318	65/80	Patient with pyelonephritis
Escherichia coli MS 69–1*	HMPREF9534_02377	EFJ81575	65/80	Human feces
Escherichia coli UTI89*	UTI89_C4964	ABE10367	65/80	Woman with uncomplicated cystitis
Escherichia coli 536	ECP_4600	ABG72536	65/80	Patient with acute pyelonephritis
Escherichia coli MS 200–1*	HMPREF9553_01535	EFJ62362	65/80	Human feces
Escherichia coli MS 153–1*	HMPREF9544_00990	EFU53892	65/80	Human feces
Escherichia coli MS 60–1*	HMPREF9533_00811	EGB84333	65/80	Human feces
Proteus mirabilis HI4320	PMI2716	CAR45338	61/78	Pathogen, urine of a nursing home patient with a long-term (>30 d) indwelling urinary catheter
Proteus mirabilis ATCC 29906*	HMPREF0693_2863	EEI47333	61/78	Urogenital tract
Providencia alcalifaciens DSM 30120*	PROVALCAL_01555	EEB46441	63/79	Human feces
Providencia rustigianii DSM 4541*	PROVRUST_06324	EFB72255	63/79	Human feces
Providencia rettgeri DSM 1131*	PROVRETT_07161	EFE54165	63/79	Human feces
Yokenella regensburgei ATCC 43003*	HMPREF0880_04646	EHM44496	61/77	Human feces collected in New Jersey
Proteus penneri ATCC 35198*	PROPEN_00404	EEG87333	62/79	Human feces, Texas

Table S3. Annotation of *cut* gene cluster in *Desulfovibrio alaskensis* G20

Gene	GenBank accession no. (protein sequence)	Size, bp/aa	Homolog in *D. desulfuricans* ATCC 27774 (% amino acid ID/similarity)	Putative function
cutA Dde_3284	ABB40078	624/208	Ddes_1355 (75/85)	Microcompartment structural protein
cutB Dde_3283	ABB40077	1506/502	Ddes_1356 (61/72)	CoA acylating aldehyde dehydrogenase
cutC Dde_3282	ABB40076	2538/846	Ddes_1357 (83/91)	Glycyl radical enzyme
cutD Dde_3281	ABB40075	933/310	Ddes_1358 (62/76)	Glycyl radical enzyme activating protein
cutE Dde_3280	ABB40074	621/207	Ddes_1359 (43/60)	Microcompartment structural protein
cutF Dde_3279	ABB40073	1458/486	Ddes_1360 (71/82)	Acetaldehyde dehydrogenase
cutG Dde_3278	ABB40072	282/94	Ddes_1361 (95/96)	Microcompartment structural protein
cutH Dde_3276	ABB40070	702/234	Ddes_1362 (65/78)	Propanediol utilization protein
cutI Dde_3275	ABB40069	822/273	Ddes_1363 (71/83)	Putative chaperonin
cutJ Dde_3274	ABB40068	591/197	Ddes_1364 (43/58)	Propanediol utilization protein
cutK Dde_3273	ABB40067	273/91	Ddes_1365 (70/81)	Microcompartment structural protein
cutL Dde_3272	ABB40066	549/182	Ddes_1366 (57/72)	Microcompartment structural protein
cutM Dde_3271	ABB40065	348/116	Ddes_1367 (41/53)	Propanediol utilization protein
cutN Dde_3270	ABB40064	276/92	Ddes_1368 (90/94)	Microcompartment structural protein
cutO Dde_3267	ABB40061	1110/369	Ddes_1369 (66/79)	Alcohol dehydrogenase
cutP Dde_3266	ABB40060	1329/442	Ddes_1370 (67/81)	NADH dehydrogenase
cutQ Dde_3265	ABB40059	552/183	Ddes_1371 (67/83)	Microcompartment structural protein
cutR Dde_3264	ABB40058	357/119	Ddes_1372 (74/88)	Microcompartment structural protein
cutS Dde_3263	ABB40057	420/140	Ddes_1373 (55/71)	Ethanolamine utilization protein

Table S4. Glycyl radical enzyme amino acid sequences used in CutC phylogenetic analysis

Gene or locus tag	Organism	GenBank accession no. (protein sequence)	Annotation
Ddes_1357	*Desulfovibrio desulfuricans* ATCC 27774	ABB40078	Choline TMA-lyase
Dde_3282	*Desulfovibrio alaskensis* G20	ABB40076	Choline TMA-lyase
CLOSTHATH_02755	*Clostridium hathewayi* DSM 13479	EFC99034	Choline TMA-lyase
CLOSPO_02864	*Clostridium sporogenes* ATCC 15579	EDU36695	Choline TMA-lyase
CTC_01449	*Clostridium tetani* E88	AAO36007	Choline TMA-lyase
HMPREF0078_1374	*Anaerococcus vaginalis* ATCC 51170	EEU12078	Choline TMA-lyase
HMPREF9553_01535	*Escherichia coli* MS 200–1	EFJ62362	Choline TMA-lyase
HMPREF0484_5138	*Klebsiella pneumoniae subsp. rhinoscleromatis* ATCC 13884	EEW38822	Choline TMA-lyase
HMPREF0693_2863	*Proteus mirabilis* ATCC 29906	EEI47333	Choline TMA-lyase
PROPEN_00404	*Proteus penneri* ATCC 35198	EEG87333	Choline TMA-lyase
dhaB1	*Clostridium butyricum*	AAM54728	Glycerol dehydratase
pflB1	*Clostridium ljungdahlii* DSM 13528	ADK14251	Glycerol dehydratase
NT01CX_1220	*Clostridium novyi* NT	ABK60580	Glycerol dehydratase
CbC4_0337	*Clostridium botulinum* BKT015925	AEB75017	Glycerol dehydratase
tutD	*Thauera aromatica*	AAC38454	Benzylsuccinate synthase
bssA	*Azoarcus* sp. T	AAK50372	Benzylsuccinate synthase
Geob_2448	*Geobacter* sp. FRC-32	ACM20801	Benzylsuccinate synthase
bssA	*Aromatoleum aromaticum* EbN1	CAI07159	Benzylsuccinate synthase
csdB	*Clostridium scatologenes*	ABB05046	4-Hydroxyphenylacetate decarboxylase
CLH_1872	*Clostridium botulinum* E3 str. Alaska E43	ACD52546	4-Hydroxyphenylacetate decarboxylase
CLL_A1723	*Clostridium botulinum* B str. Eklund 17B	ACD24941	4-Hydroxyphenylacetate decarboxylase
hpdB	*Clostridium difficile* ATCC 9689	CAD65889	4-Hydroxyphenylacetate decarboxylase
pflB	*Escherichia coli* str. K-12 substr. MG1655	AAC73989	Pyruvate formate-lyase
SARI_01990	*Salmonella enterica subsp. arizonae*	ABX21870	Pyruvate formate-lyase
pflB	*Klebsiella pneumoniae subsp. pneumoniae* MGH 78578	ABR76367	Pyruvate formate-lyase
pfl1	*Photorhabdus luminescens subsp. laumondii* TTO1	CAY33841	Pyruvate formate-lyase
CGSHiGG_03495	*Haemophilus influenzae* PittGG	ABQ99687	Pyruvate formate-lyase
VIBHAR_01546	*Vibrio harveyi* ATCC BAA-1116	ABU70516	Pyruvate formate-lyase
pflB	*Clostridium butyricum* 5521	EDT76498	Pyruvate formate-lyase

Table S5. Primers used for cloning and site directed mutagenesis

Oligo	Sequence	Restriction site
Dde-3282-NdeI-start	5′ GCATCATATGGATCTCCAGGACTTTTCACATAAGC 3′	NdeI
Dde-3282-XhoI-nostop	5′ GATTCTCGAGGAAACCATGCAGCATGG 3′	XhoI
Dde-3281-NdeI-start	5′ GCATCATATGAGAACCGCAACACACAGAGACG 3′	NdeI
Dde-3281-XhoI-stop	5′ GATTCTCGAGTCAGTGGCGGATCACCGAAACC 3′	XhoI
Dde3282-QC-C489A-for	5′ GACTACTGCCTGATGGGTGCCGTGGAACCGCAG 3′	—
Dde3282-QC-C489A-rev	5′ CTGCGGTTCCACGGCACCCATCAGGCAGTAGTC 3′	—
Dde3282-QC-G821A-for	5′ GTGGTGCGCGTGGCCGCATACAGCGCCTTCTTC 3′	—
Dde3282-QC-G821A-rev	5′ GAAGAAGGCGCTGTATGCGGCCACGCGCACCAC 3′	—