# Supplementary Information

# Refined Experimental Annotation Reveals Conserved Corrinoid Autotrophy in Chloroform-Respiring *Dehalobacter* Isolates

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**Note:** Please also refer to Table S1 of our previous publication (http://journal.frontiersin.org/file/downloadfile/173415_supplementary-materials_tables_1_xls/octet-stream/Table%201.XLS/323/1/173415) in *Frontiers in Microbiology* (http://journal.frontiersin.org/article/10.3389/fmicb.2016.00100/full) contains classified and curated genome annotations of 5 *Dehalobacter* strains compared *Dehalococcoides mccartyi* Strain 195 and *Desulfitobacterium hafniense* strain Y51.

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## References

**Appendix:** Complete DNA sequence and protein sequence of recombinant serine hydroxymethyltransferase from strain CF (amino acids in bold represent residues added for the N-terminal $His_6$-tag)

# Supplementary Materials and Methods

All Chemicals were ordered from Sigma-Aldrich at highest purity available unless specified otherwise.

## 1.0 Detailed medium recipes

**(i) Vitamin solution.** The core vitamin solution used in this study contains 2 µg/L folic acid, 5 µg/L α-lipoic acid, 2.5 µg/L nicotinic acid, 2.5 µg/L nicotinamide, 5 µg/L 4-aminobenzoic acid, 5 µg/L pantothenic acid, 2.5 µg/L pyridoxine, 2.5 µg/L pyridoxal amine, 5 µg/L riboflavin. The following vitamins: 20 µg/L biotin, 25 µg/L cyanocobalamin, 25 µg/L thiamine, or 25 µg/L heme (hematin form) were added to the medium where specified in the main text. Heme was first dissolved in 10 mM NaOH to a concentration of 25 mg/L (1,000-fold stock).

**(ii) Mixed culture supernatant (MCS).** Autoclaved sterile ACT-3 MCS was added to medium to support the growth of the two *Dehalobacter* strains in dilution-to-extinction transfers. To prepare MCS, mixed culture ACT-3 was harvested when the culture was steadily dechlorinating 1,1,1-trichloroethane (1,1,1-TCA) to CA (mono-chloroethane), and was centrifuged at 9,000 ×g at 4 °C for 20 min under anaerobic conditions. The supernatant was then filter-sterilized using a Millipore 0.22 µm filter and stored at -20 °C until use.

**(iii) Amorphous iron sulfide (FeS) slurry.** In an anaerobic chamber, equimolar amounts $Na_2S$ (0.78 g) and $FeCl_2$ (1.27 g)were added to a 160 mL narrow neck serum bottle, and suspended in 100 mL anaerobic Milli-Q water to a final concentration of 100 mM. The bottle was sealed with a butyl rubber stopper and crimped. After sparging with $N_2$ for 20 minutes to get rid of most the $H_2S$, the black FeS suspension was transferred under anaerobic conditions to a 250 mL polypropylene centrifuge tube (Thermo) and centrifuged at 8,000 x g for 10 min at RT. The supernantant was removed and the slurry resuspended in anaerobic Milli-Q water again. The step was repeated once and the resuspension was transferred back to the serum bottle. The bottle was sealed by a butyl rubber stopper, crimped, and autoclave-sterilized. The final concentration of the FeS slurry was approximately (~8 g/L).

## 2.0 Growth conditions for growth assays of electron donors, electron acceptors, and vitamin requirements

Briefly, 10 mL of minimal mineral medium, main carbon source (5 mM), electron donor (10 mM) (or 6.4 mL of $H_2$), 1-4 µL of neat chlorinated substrates, and each of L-arginine, L-histidine, and L-threonine (0.1-0.5 mM) were added to a 25 mL Bellco tube before autoclaving. Acetate, $H_2$, and 1,1,1-TCA or 1,1-DCA were used to grow the isolate cultures in most experiments unless specified otherwise. After autoclaving, 10 µL sterile FeS slurry and vitamin solution (from 1,000-fold stock) were injected to the sealed tubes outside the anaerobic chamber. The serial transfer assays were inoculated with 1 mL of culture ($10^{-1}$ dilution). The nutrients requirement assays were inoculated with 100 µL of culture ($10^{-2}$ dilution). To minimize carryover, inocula were pelleted down to remove supernatant, and were resuspended in and equal volume of sterile minimal mineral medium before inoculation. For large-scaled cultivation (1 L) for corrinoid structure characterization by UPLC-MS and the formate utilization assay, casamino acid (0.5 g/L) (Fisher Scientific) was supplemented to medium.

## 3.0 PCR and Restriction fragment length polymorphism (RFLP)

To amplify 16S rRNA gene sequences for RFLP analysis and *cfrA/dcrA* for purity test, DNA samples from *Dehalobacter* isolates and mixed culture ACT-3 were extracted using the MoBio UltraClean® Microbial DNA Isolation Kit. In the PCR reactions, a Thermo PCR master mix was used and the thermocycling program was as follows: (i) for 16S rRNA gene amplification, initial denaturation of 10 min at 95 °C; 40 cycles of 30 s denaturation at 95 °C, 30 s annealing at 50 °C and 2 min extension at 72 °C; and final extension of 10 min at 72 °C; (ii) for *cfrA/dcrA* amplification, initial denaturation of 10 min at 95 °C; 40 cycles of 30 s denaturation at 95 °C, 30 s annealing at 60 °C and 30 s extension at 72 °C; and final extension of 10 min at 72 °C. General bacterial 16S rRNA primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1541r (5'-AAGGAGGTGATCCAGCCGCA-3') were used in PCR reactions for RFLP analysis (Löffler *et al.*, 2000). The PCR-amplified 16S rRNA gene product (1 µg) was then digested for 1 h using restriction enzymes NheI-HF and XbaI-HF at 37ºC according to the manufacturer's standard protocol. Fragments were resolved by electrophoresis for 30 min on 1% agarose gels (100 V). Specific primers 413f (5'-CCCGAACCTCTAGCACTTGTAG-3') and 531r (5'-ACGGCAAAGCTTGC ACGA-3') for *cfrA* as well as 424f (5'-AGCACTCAGAGAGC

GTTTTGC-3') and 533r (5'-CAACGGCCCAGCTTGCAT-3') for *dcrA* were used in the PCR reactions for purity test of potential cross-contamination of two *Dehalobacter* strains in their isolate cultures. Fragments were resolved by electrophoresis for 25 min on 2.5 % agarose gels (90 V).

## 4.0 Microscopy

For epifluorescence microscopy, 0.1 mL culture samples were centrifuged at 13,000 g for 10 min at room temperature to pellet cells. The pellets were resuspended in 5 µL Milli-Q water and the suspension pipetted onto glass slides for DAPI or Gram-stain. Slides were air-dried, flame-fixed, and stained with DAPI (10 µg/mL) for 10 min. After washing slides to remove stain, a droplet of immersion oil was loaded on each slide and the slides were observed using a epifluorescent microscope (BX 51, Olympus) with a 100x UPlan Apochromat objective, 150 W xenon lamp (Opti Quip), a blue excitation filter cube (excitation band pass 372 nm; emission barrier filter 456 nm) and 10x focusing eyepiece to check for uniformity of cell morphology (1,000x magnification). Gram-stain was performed using a commercial kit (Sigma-Aldrich).

Preparation of samples for Scanning and Transmission Electron Microscopy (SEM and TEM) was performed using the service of the Advanced Bioimaging Centre of Mount Sinai Hospital (Ontario, Canada). For scanning electron microscopy (SEM) observation, the pellets were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 2 h and rinsed in buffer. The sample was then dehydrated in an ethanol gradient series and followed by a critical point drying in a Bal-tec CPD030 critical point dryer. After the drying, the sample was mounted on aluminum stub and coated with gold in a Denton Desk II sputter coater and examined in a Hitachi SU8230 cold FEG SEM at 5 kV and image processing was performed using the service of Ontario Centre for Characterization of Advanced Materials (OCCAM) of University of Toronto. Samples for transmission electron microscopy (TEM) were fixed in 2% glutaraldehyde in the same sodium cacodylate buffer, rinsed in buffer, and then post-fixed in 1% osmium tetroxide in buffer. After fixation, samples were dehydrated in an ethanol gradient series followed by a propylene oxide treatment. Subsequently, samples were embedded in EMBed 812 resin, cut on an RMC MT6000 ultramicrotome and stained with uranyl acetate and lead citrate. TEM was carried out using a Hitachi HF-3300 at 200 kV and image processing was performed using the service of Ontario Centre for Characterization of Advanced Materials (OCCAM) of University of Toronto.

## 5.0 Enzyme activity assays

The preparation of cell extracts was conducted under aerobic conditions except for the RDase activity assays. Fifty milliliters of *E. coli* strain K-12 culture grown on LB medium or 200 mL of strain CF culture grown on 1,1,1-TCA were harvested, ice chilled, and centrifuged at 9,000 x g for 20 min at 4ºC. The pellets were resuspended in 0.5 mL of 100 mM Tris-HCl buffer (pH 7.5) containing 12.5 mM NaCl, 2.5% glycerol, and 1% digitonin. The suspensions were loaded into a 2 mL O-ring-capped plastic microcentrifuge tube containing 50 µL in volume of 0.1 mm diameter glass beads. To lyse the cell, the tube was vortexed in maximum intensity for 2 min and incubated on ice for 1 min. The step was repeated 4 times. The cell lysates were then centrifuged at 20,000 x g for 15 min at 4ºC to pellet down the cell debris, beads, and unbroken cells.

**(i) RDase activity assay.** Cell extracts were loaded into 2 mL glass vials which contains 1 mL of 25 mM Tris-HCl buffer (pH 7.5), 2 mM titanium citrate, 2 mM methyl viologen, and 1 mM chlorinated substrates (nominal concentration). The vials were capped, incubated upside down at RT for 24 h and sampled for GC analysis as previously described (Tang and Edwards, 2013).

**(ii) Malate dehydrogenase (MDH) activity assay**. The MDH activity assays were conducted following an established protocol (Lin *et al.*, 2015) with some modifications. Twenty five micro liters of cell extracts were loaded into each well of Falcon 96-well microplate which contains 200 µl of 25 mM potassium phosphate buffer (pH 7.5), 1 mM NADH, 0.1 mM EDTA, and 0.1 mM $MgCl_2$. The mixtures were first incubated at 30$^{o}$C for 1 min, and 25 µl of 10 mM sodium oxaloacetate was then loaded into each well to a final concentration of 1 mM. The assays were incubated at 30$^{o}$C for 10 min. The decrease of absorbance at 340 nm (where NADH absorbs) in each assay was recorded. The assay was calibrated with freshly prepared NADH standards at 340 nm.

**(iii) NADP dependent malate enzyme (NDME) activity assay.** The conditions of NDME activity assays (Geer *et al.*, 1980) were similar to MDH assay with the following modifications. NADP replaced NADH in the assay buffer. Sodium malate (50 mM) was replaced sodium oxaloacetate to a final concentration of 5 mM in each assay. The increase of absorbance at 340 nm was recorded and the assay was calibrated with freshly prepared NADPH standards at 340 nm.

**(iv) Succinate dehydrogenase (SDH) activity assay:** The conditions for the SDH activity assays were similar to MDH with the following modifications. Sodium azide (2 mM) (electron transport chain inhibitor), 2,6-dichlorophenolindophenol (DCPIP) (0.2 mM), and phenazine methosulfate (0.2 mM) replaced NADH in the assay buffer (Kolaj-Robin *et al.*, 2011). Sodium succinate replaced oxaloacetic acid to a final concentration of 1 mM. The decrease of absorbance at 600 nm in each assay was recorded reflecting reduction of DCPIP. The assay was calibrated with freshly prepared DCPIP standards. When oxidized, DCPIP is blue with a maximal absorption at 600 nm; when reduced, DCPIP is colorless.

**Serine hydroxymethyltransferase (SHMT)** and **threonine aldolase activity** assays were conducted using the purified recombinant SHMT from strain CF. Methods for cloning, expression, and purification of SHMT are described below and the nucleotide and protein sequences are also provided in Appendix.

**(v) SHMT activity assay.** SHMT activity assays were conducted in 0.25 mL reaction mixtures by adding 1 mM L-serine, 1 mM mercaptoethanol, 2 mM tetrahydrofolate (THF), 0.25 mM pyridoxal phosphate (PLP), and 50 µg/mL purified SHMT (Buchenau and Thauer, 2004, Ogawa *et al.*, 2000). Two controls were also prepared, one without enzyme and the other without serine. All mixtures were incubated at 30$^{o}$C for 75 min. SHMT activities were determined by measuring the formation of glycine from L-serine using UPLC-ESI-MS as described below.

**(vi) Threonine aldolase activity assay.** Threonine aldolase activity assays were conducted in 1 mL reaction mixtures by adding 20 mM L-threonine (Sigma-Aldrich), 50 µM of PLP, 1 mM DTT, and 50 µg of purified SHMT to 50 mM phosphate buffer (pH 7.3) (Buchenau and Thauer, 2004, Ogawa *et al.*, 2000). Two controls were also prepared, one without enzyme and the other without threonine. Threonine aldolase activities were determined by measuring the formation of acetaldehyde from L-threonine using HPLC.

## 6.0 Cloning of SHMT

The SHMT gene (locus tag: DCF50_p2888) from *Dehalobacter* sp. strain CF was cloned into pCOLADUET$^{TM}$-1 vector (Novagen), between BamHI and PstI restriction sites in the MCS1 cloning site, which encodes an N-terminal $His_6$-tag. SHMT was amplified with primers

F_SHMT_BamHI (5'-AGTCATTGGATCCAATGGATTACATTCGGAAATATTTAGC-3',) and R_SHMT_PstI (5'-AGTCATTCTGCAGTTACTTATACAAAGGAAATCTTCC-3'). The standard protocol for Q5® High Fidelity DNA polymerase (New England BioLabs® Inc.) was used, but with an annealing temperature of 58$^{o}$C and elongation time of 39 seconds. After amplification and purification of the PCR product, 1 µg of amplicon and empty pCOLADUET-1 empty vector were each digested with BamHI-HF (New England BioLabs® Inc.) and PstI-HF (New England BioLabs® Inc.) by incubating at 37$^{o}$C for 1 h. After clean-up using a PCR purification kit (Thermo Scientific), the digested vector was dephosphorylated using Shrimp Alkaline Phosphatase (New England BioLabs® Inc.), while the digested amplicon was phosphorylated using T4 polynucleotide kinase (New England BioLabs® Inc.). Ligation was carried out using T4 DNA ligase (New England BioLabs® Inc.) using a 3:1 molar ratio of insert to plasmid and incubating the mixture at 16$^{o}$C overnight. Chemically-competent *E. coli* strain DH10β cells were transformed with 5 µL of the ligation mixture. The gene sequence was verified by Sanger sequencing. The sequence verified plasmid was then transformed into the expression host, chemically-competent *E. coli* strain BL21 (DE3) Gold (Stratagene). DNA (5'-3') and protein sequences of recombinant SHMT from strain CF are provided at the end of this file.

**7.0 Expression and purification of SHMT.** *E. coli* strain BL21 carrying the SHMT expression vector was first grown in 10 mL LB medium (BioShop Inc.) with 50 µg/mL of kanamycin, at 37$^{o}$C overnight. The seed culture was then transferred to 1 L of terrific broth with 50 µg/mL of kanamycin in a 2.5 L baffled flask, and grown at 37$^{o}$C. When the $OD_{600\ nm}$ of the culture reached 0.1, the temperature was lowered to 16$^{o}$C. After another 3 h of shaking, protein expression was induced by the addition of IPTG (to a final concentration of 1 mM) to the culture which continued growing overnight. The cells were harvested at 4$^{o}$C and resuspended in binding buffer containing 50 µM pyridoxal phosphate (PLP). The resuspended cells were sonicated for 20 min (program 3 s on and 4 s off) in an ice-water bath. The resulting suspension was centrifuged at 58,000 xg for 40 min at 4 $^{o}$C. The supernatant was loaded onto a Ni-NTA column that was equilibrated with 50 mM HEPES buffer (pH 7.5) with 0.5 M NaCl, 10% glycerol, 50 µM PLP, and 5 mM imidazole, and washed with 150 mL the same buffer with 30 mM imidazole. The protein is then eluted using the same buffer with 250 mM imidazole and fractions were monitored for protein concentration using standard Bradford assay. The protein purity was verified by SDS-PAGE (Figure S5).

## 8.0 Extraction and derivatization of intracellular thiamine species

In brief, 200 mL of a stationary-phase strain CF culture grown on thiamine-containing (25 μg/L) medium was centrifuged at 9,000 x g for 10 min at 4°C. Cell pellet (50 mg wet weight) or 10 μL of 0.1 mM thiamine standards (to a final concentration of 1 μM) were suspended and diluted in 1 mL of 3.6% perchloric acid (w/v), respectively, followed by a 20 min ice-chilled water bath on a ultrasonic cleaner. The cell lysate was then centrifuged at 16,000 x g for 15 min at 4°C. To derivatize the thiamine species, supernatant (0.5 mL) was mixed with a $K_3[Fe(CN)_6]$ (12 mM in 3.35 M NaOH) solution (0.1 mL). The mixtures were incubated in the dark at 37°C for 15 min, and then adjusted to pH 7 with 85% phosphoric acid (w/v) for later analysis.

## 9.0 Analytical procedures

Chlorinated hydrocarbons were measured by injecting a 0.3 mL headspace sample into a Hewlett-Packard 5890 Series II gas chromatograph (GC) fitted with a GSQ column (30-m-by-0.53-mm [inner diameter] PLOT column; J&W Scientific, Folsom, CA), as described previously (Grostern and Edwards, 2006). The GC carrier gas pressure was initially 100 kPa, and the oven temperature was programmed to hold at 50°C for 90 s and then increase to 155°C at 30°C/min and then increase to 180°C at 4°C/min and hold for 5 min. Calibration was with external standards.

The amount of acetaldehyde produced from the threonine aldolase activity assay was determined by HPLC using a Dionex Ultimate 3000 system equipped with an Aminex HPX-87H column (BioRad) connected to a UV detector. Twenty microliters of each sample was injected onto the column incubated at 60$^{o}$C, using 5 mM $H_2SO_4$ eluent at a flow rate of 0.6 mL/min with the UV wavelength set to 278 nm for acetaldehyde detection.

Thiamine derivatives were detected by high pressure liquid chromatography (HPLC)-Fluorescence using a Varian Prostar HPLC system consisting of a Model 230 solvent delivery system, a Model 410 AutoSampler, a Model 500 column valve module, and a Model 363 Fluorescence detector (excitation at 365 nm and emission at 435 nm). Separation was performed using a 5 μm Varian Pursuit C18 column (15 cm x 4.6 mm) with a Varian MetaGuard guard column. The mobile phase consisted of two eluants: Mobile phase A was dibasic sodium

phosphate (25 mM, pH 7.0):methanol (90:10, v/v); mobile phase B was dibasic sodium phosphate (25 mM, pH 7.0):methanol (30:70, v/v). To enhance the fluorescence intensity, sodium perchlorate (Sigma-Aldrich) was added to both solvents to a final concentration of 1 mM. Gradient steps were programmed as follows: 0 min, 0% B; 4.5 min, 13% B; 10 min, 50% B; 15.5 min, 50% B; 19 min, 0% B; 24.5 min, 0% B, with a flow rate of 0.85 mL/min. Injection volume were 20 µL.

Corrinoids and serine were detected and quantified using Liquid chromatography coupled mass spectrometry (LC-MS) with an Accela HPLC system and an Exactive mass spectrometer (Thermo Scientific), while heme (hematin form) and biotin detection was performed using a Dionex Ultimate 3000 UPLC and a Q-Exactive mass spectrometer, both equipped with HESI II sources (Thermo Scientific). System control and data handling were performed using Thermo XCalibur 2.2 software. Separation by liquid chromatography was conducted on a Hypersil Gold C-18 column (50mm x 2.1 mm, 1.9 µm particle size, Thermo Scientific) equipped with a guard column. LC was performed with 10 uL injections at a flow rate of 0.2 ml/min with a gradient of water containing 2.5 mM ammonium acetate (pH 6.0) (A) into methanol (B), and a column temperature of 30˚C. The gradient was 0 min, 0% B; 0.46 min, 0% B; 0.81 min, 15% B; 3.32 min, 50% B; 4.86 min, 90% B; 5.56 min, 90% B; 5.59 min, 0% B; followed by equilibration for 5 min with 0% B. Corrinoid data collection was done in positive ionization mode with a *m/z* scan range of 50-150 and 1000-1500 for serine and corrinoid, respectively; resolution 100,000 at 1 Hz, automatic gain control (AGC) target of 5e5; and a maximum injection time of 200 ms. Heme data collection was done in negative ionization mode with a *m/z* scan range 600-800; resolution 140,000 at 1Hz; AGC target 3e6; and a maximum injection time of 250 ms. Injection volume for heme is 25 µL. Biotin was detected with a different mobile phase with a gradient of water containing 20 mM ammonium acetate and 20mM ammonium hydroxide (A) into acetonitrile (B). The gradient was 0 min, 0% B; 6 min, 100% B; 8 min, 0% B; followed by equilibration for 4 min with 0% B. Biotin data collection was done in positive ionization mode with a *m/z* scan range of 100-600; resolution 70,000 at 1 Hz, automatic gain control (AGC) target of 3e6; and a maximum injection time of 250 ms. Injection volumes for biotin detection were 20 µL.

# Supplementary Tables

**Table S1A.** Electron donors tested with strains CF and DCA

| Electron donor | Dechlorination [a] |
|---|---|
| **Methanol** | - |
| **Ethanol** | - |
| **Formate** | +[b] |
| **Acetate** | - |
| **Propionate** | - |
| **Pyruvate** | - |
| **Lactate** | - |
| **Glucose** | - |
| **$H_2$** | + |

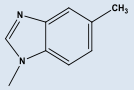
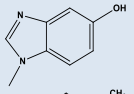
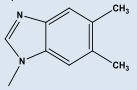
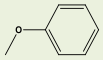
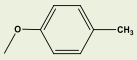
[a] "+" represents the detection of sustained dechlorination of 1,1,1-TCA or 1,1-DCA after two transfers into chemically defined medium. Transfers were made using a 10% dilution. Before inoculation, cells were pelleted down to remove the supernatant, and then were suspended in sterile mineral medium.

[b] Formate utilization by strains CF and DCA requires the supplementation of either ACT-3 mixed culture supernatant, yeast extract, or casamino acids.

**Table S1B.** Electron acceptors tested with strains CF and DCA

| | Strain CF | | Strain DCA | |
|---|---|---|---|---|
| **e- acceptor** | **Dechlorination** | **Product** | **Dechlorination** | **Product** |
| **CF** | + | **DCM** | - | |
| **DCM** | - | | - | |
| **1,1,1-TCA** | + | **1,1-DCA** | - | |
| **1,1-DCA** | - | | + | **CA** |
| **1,1,2-TCA** | + | **1,2-DCA (major) VC (minor)** | + | **1,2-DCA (major) VC (minor)** |
| **1,2-DCA** | - | | - | |
| **CA** | - | | - | |
| **PCE** | - | | - | |
| **TCE** | - | | - | |
| **VC** | - | | - | |
| **1,1-DCE** | - | | - | |

**Table S2: Chemical properties of the benzimidazoyl and phenyl cobamide standards in UPLC-ESI-MS**

| Group | Corrinoid | Theoretical molecular weight | *m/z* value from ESI-LC-MS | Lower ligand | Structure of lower ligand | Molecular wieght | Retention Time (min) |
|---|---|---|---|---|---|---|---|
| Benzimidazole | Cobinamide (Cbi) | 1015.27 | 1015.4909 | N/A | N/A | N/A | 8.51 |
| | No specific name | 1327.32 | 1327.5422 | Benzimidazole (Ben) | | 118.14 | 8.12 |
| | No specific name | 1341.34 | 1341.5588 | 5'-Methylbenzimidazole (5'-MeBen) |  | 132.16 | 8.15 |
| | Factor III | 1343.32 | 1343.5378 | 5'-Hydroxybenzimidazole (5'-OHBen) |  | 134.14 | 8.1 |
| | Vitamin $B_{12}$ (Cobalamin) | 1355.37 | 1355.5739 | 5',6'-Dimethylbenzimidazole (DMB) |  | 146.19 | 8.21 |
| | Factor $III_m$ | 1357.34 | 1357.5525 | 5'-Methoxybenzimidazole (5'-MeOBen) | | 148.16 | 8.14 |
| Phenol | No specific name | 1303.29 | 1303.5313 | Phenol |  | 94.11 | 8.96 |
| | No specific name | 1317.32 | 1317.5465 | 4'-Methylphenol (*p*-Cresol) |  | 108.14 | 9.19 |

# Supplementary Figures

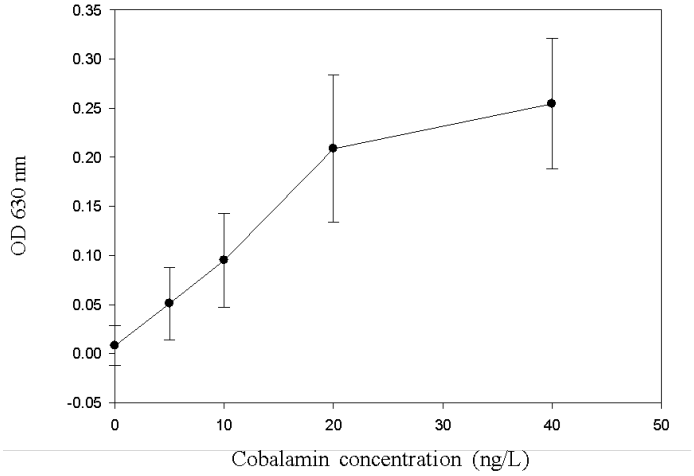



**Figure S1. Growth of *Lactobacillus delbrueckii* (ATCC=7831) measured at $OD_{630\ nm}$ in response to various concentrations of cobalamin.** Data are means ± SE of five replicates in each experiment.

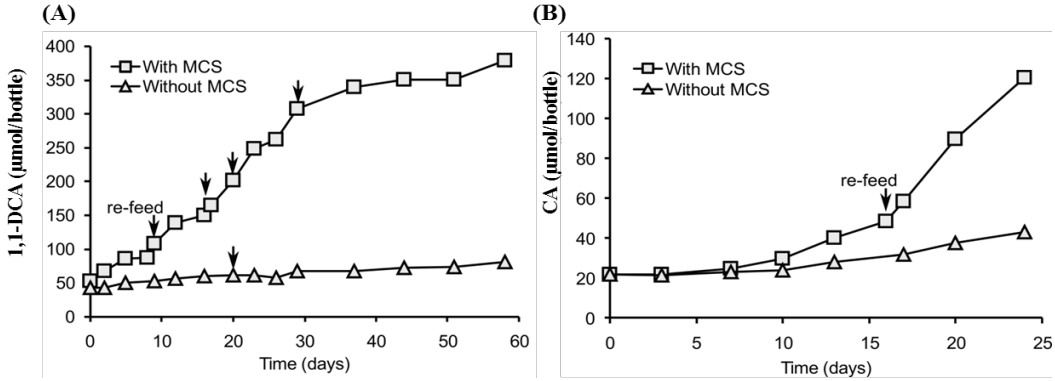



**Figure S2. Typical cumulative dechlorination profiles of $10^{-9}$ dilution-to-extinction transfers of strains (A) CF and (B) DCA.** The black arrows represent the times when chlorinated substrates we refed. Before adding a new dose of substrates, the cultures were purged with $N_2/CO_2$ (80% v/v) to remove dechlorinated products. Symbols: CA, monochloroethane; 1,1-DCA, 1,1-dichloroethane.

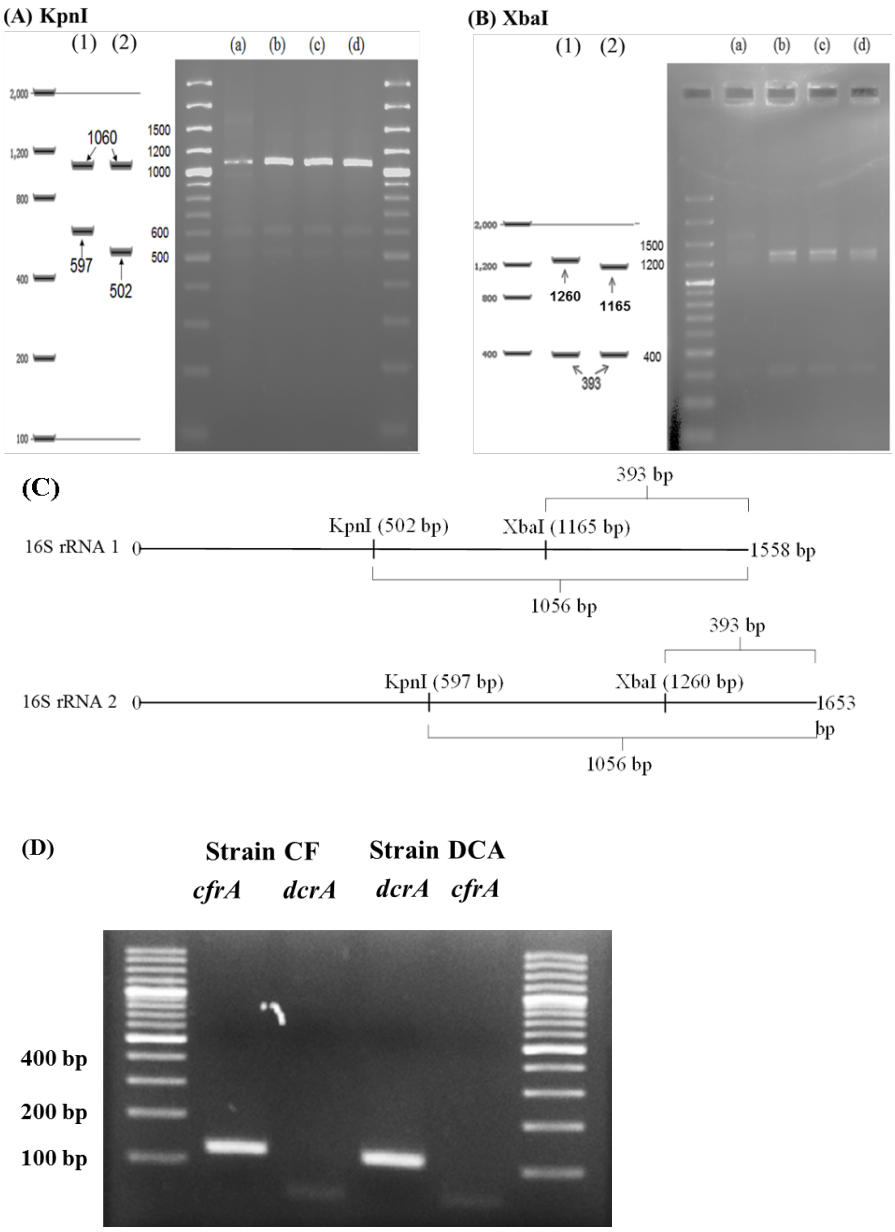



**Figure S3. PCR amplification of 16S rRNA genes and *cfrA*/*dcrA* from strains CF and DCA isolate cultures.** Restriction digestion analysis with (A) KpnI and (B) XbaI: (a) ACT-3 mixed culture; (b) strain CF isolate grown in medium with autoclaved ACT-3 mixed culture supernatant; (c) strain CF isolate grown on defined medium with complete vitamin supplement; (d) strain CF isolate grown in defined medium without biotin, cobalamin, and thiamine. (C) predicted restriction sites and lengths of the two 16S rRNA gene sequences (5'-3') and their digested fragments; (D) examination of potential cross-strain contamination in strain CF and DCA DNA using specific primers of *cfrA*/*dcrA*. The size of *cfrA* and *dcrA* amplicons is 119 bp and 110 bp, respectively. Note that the two bands below 100 bp are primers.

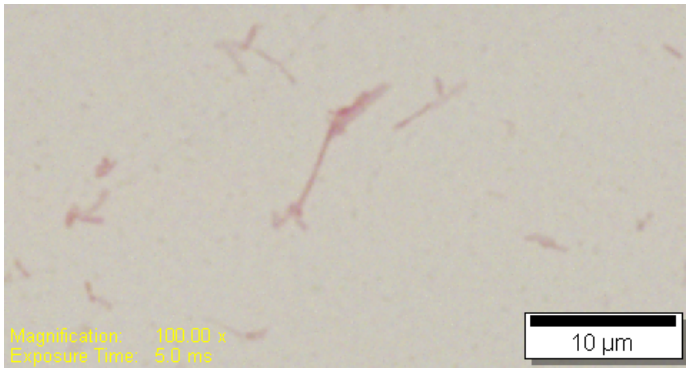



**Figure S4. Gram-stain microscopic image of *Dehalobacter restrictus* strain CF cells.**

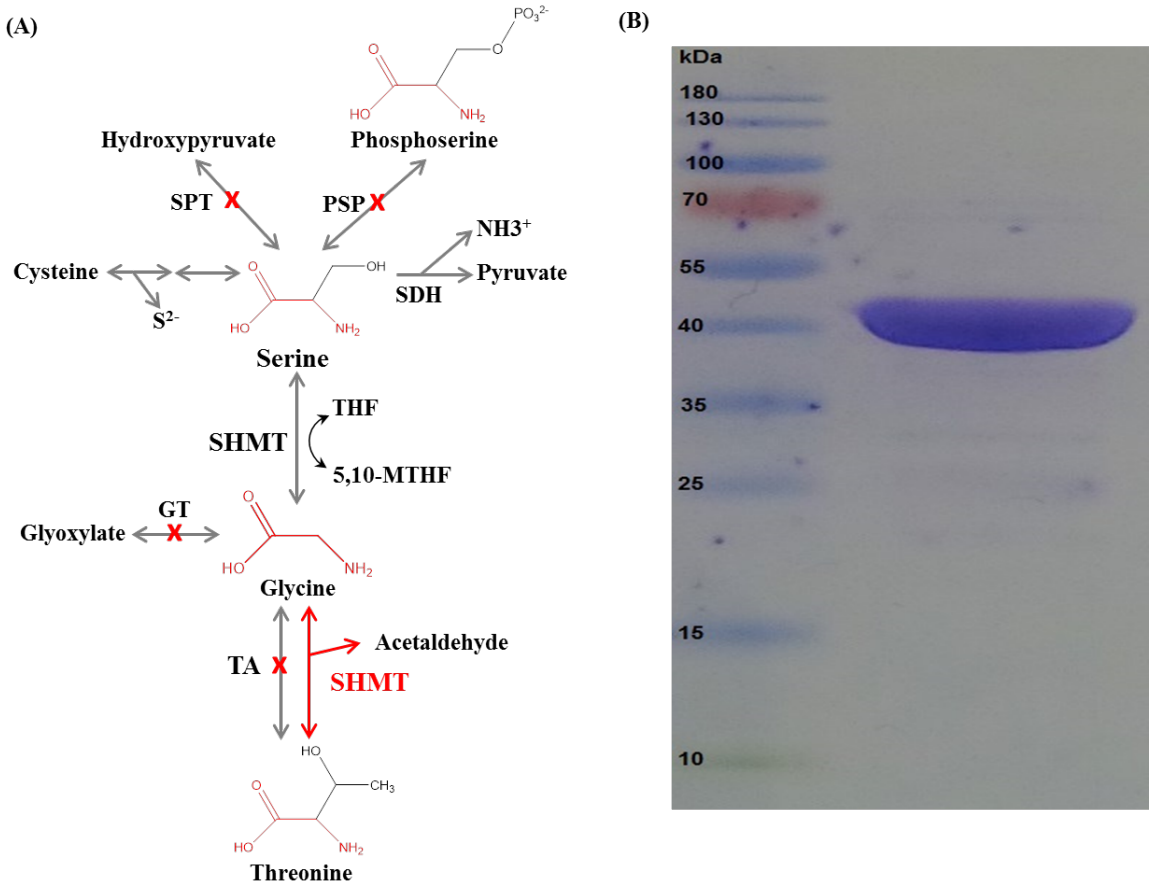



**Figure S5. Serine salvage from threonine via glycine in *Dehalobacter restrictus* strain CF.** (A) Schematic of serine biosynthesis in strain CF; (B) purified recombinant *Dehalobacter* serine hydroxymethyltransferase (42 KDa). Abbreviations: GT, glycine transaminase; PSP, phosphoserine phosphatase; SDH, serine dehydratase; SPT, serine-pyruvate transaminase; THF, tetrahydrofolate; 5,10-MTHF, 5,10-methylenetetrahydrofolate.

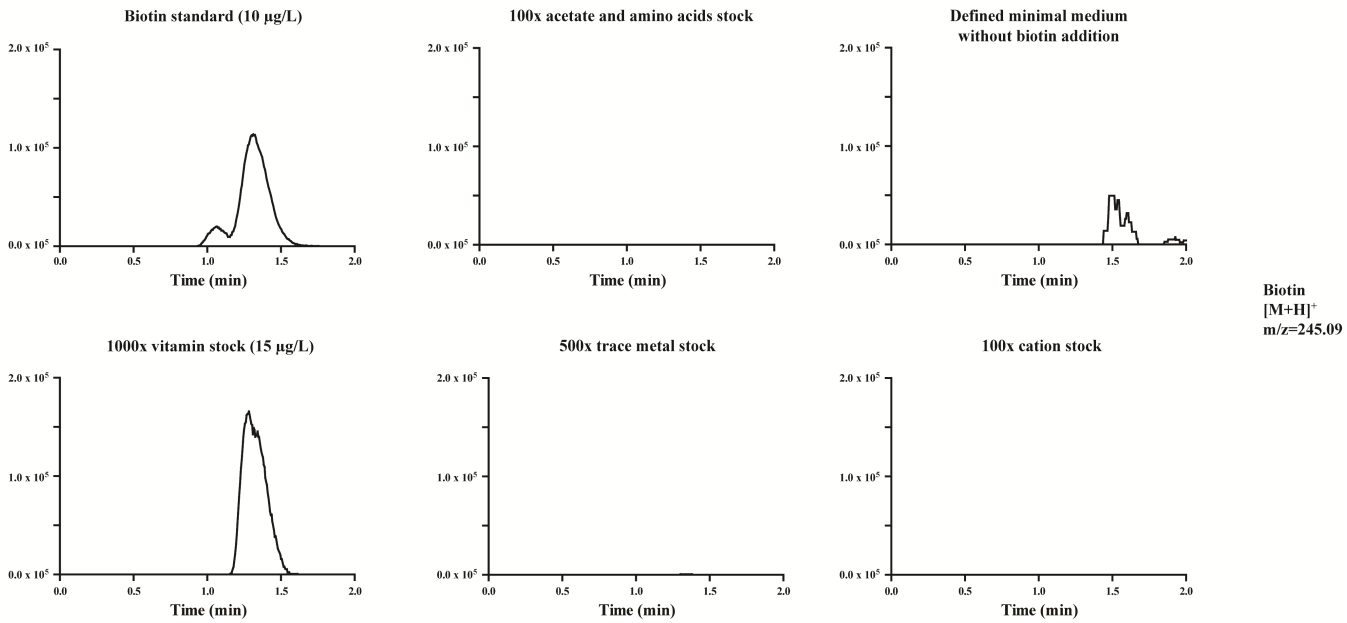



**Figure S6. MS detection of potential biotin (m/z=245.09) in the stock solutions used to make the defined medium (without biotin added) for strain CF growth (injection volume: 20 µL).** The six chromatograms are extracted ion chromatograms (m/z=245.09 ± 0.002); chemicals are ionized in positive mode. The detection limit in the LC-MS method is 1 µg/L (0.02 ng) with a linear range from 5 to 50 µg/L.

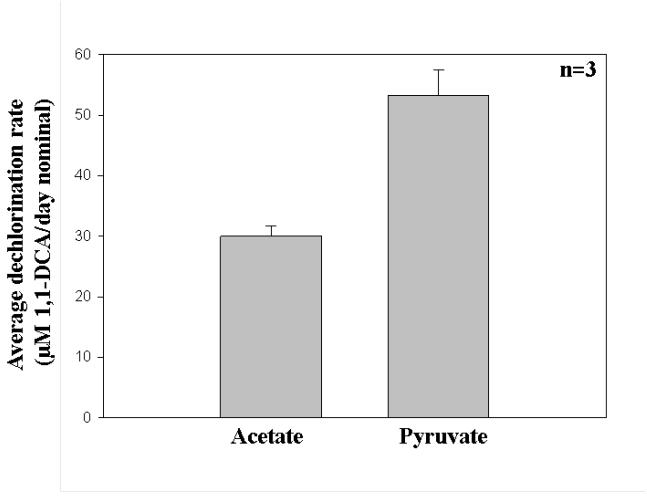



**Figure S7. Average dechlorination rates of strain CF grown on acetate or pyruvate as the main carbon source with 0.1 mM of arginine, histidine, and threonine.** Symbols: ♦, 1,1-DCA. Data are means ± SE of three replicates in the representative experiment.

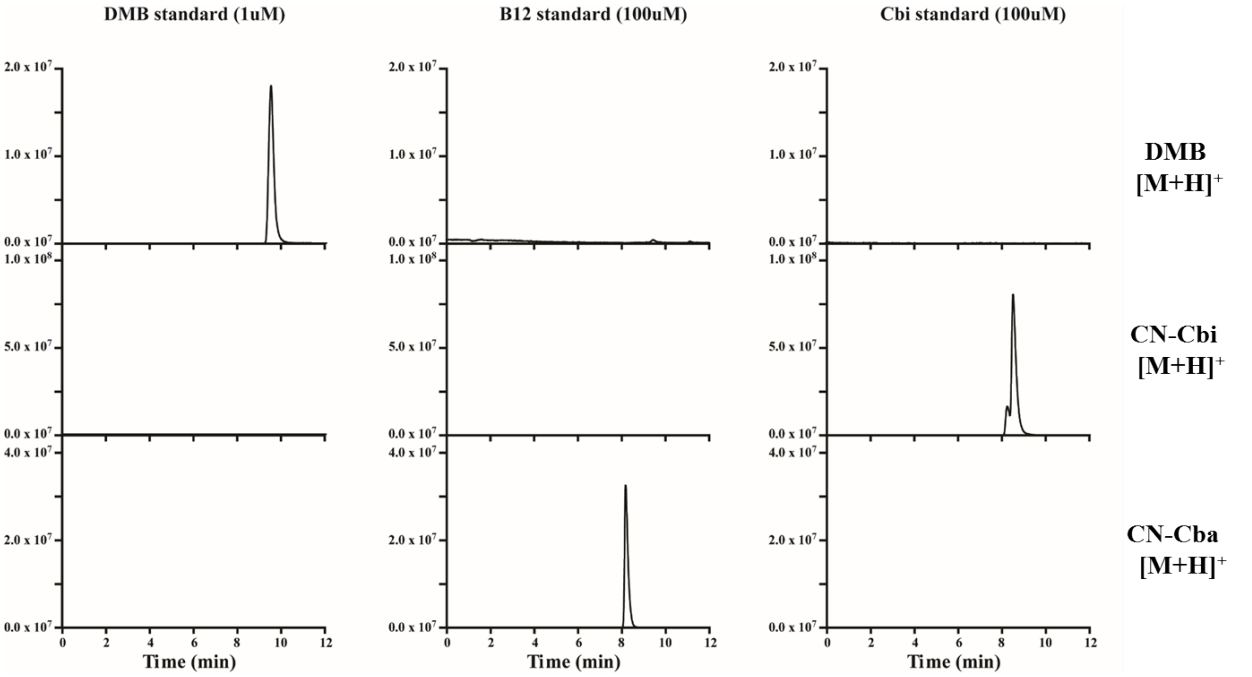



**Figure S8. MS detection of potential cyanocobinamide (CN-Cbi; m/z=1015.49) and cyanocobalamin (CN-Cba; m/z=1355.57) in the DMB standard (m/z=147.09) used for strain CF growth (injection volume: 25 µL).** The chromatogram on top panel shown are extracted ion chromatograms and the chemicals are ionized in positive mode.

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**Appendix: Complete DNA sequence and protein sequence of recombinant serine hydroxymethyltransferase from strain CF (amino acids in bold represent residues added by the N-terminal $His_6$-tag)**

5'-

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RFPLY