Geobacter sp. strain IAE dihaloeliminates 1,1,2-trichloroethane and 1,2-dichloroethane

Lisi Jiang [1 †,‡], Yi Yang [1 †], Huijuan Jin [†,‡], Hongyan Wang [†,‡], Cynthia M. Swift [#], Yongchao Xie [§], Torsten Schubert [¶], Frank E. Löffler [#,§,◊,□,×], and Jun Yan [*†]

[†] Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences; Shenyang, Liaoning 110016, China; [‡] University of Chinese Academy of Sciences, Beijing, China, 100049; [#] Center for Environmental Biotechnology, University of Tennessee, Knoxville, Tennessee 37996, USA; [§] Department of Civil and Environmental Engineering, University of Tennessee, Knoxville, Tennessee 37996, USA; [¶] Research Group Anaerobic Microbiology, Friedrich Schiller University, Jena, Germany. [◊] Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37996, USA; [□] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA; [×] Department of Biosystems Engineering & Soil Science, University of Tennessee, Knoxville, Tennessee 37996, USA.

[1] L.J. and Y.Y. contributed equally to this study

∗ Corresponding author

Jun Yan, Institute of Applied Ecology, Chinese Academy of Sciences, 512 South Building, 72 Wenhua Road, Shenyang, Liaoning 110016, China, Phone: +86-2483970426, E-mail: junyan@iae.ac.cn

Running title: Microbial dihaloelimination of 1,1,2-TCA and 1,2-DCA

ORCID

Yi Yang: 0000-0002-3519-5472

Yongchao Xie: 0000-0001-5112-0553

Torsten Schubert: 0000-0002-6569-1194

Frank Löffler: 0000-0002-9797-4279

Jun Yan: 0000-0001-6883-8529

Abstract

Chlorinated ethanes, including 1,2-dichloroethane (1,2-DCA) and 1,1,2-trichloroethane (1,1,2-TCA), are widespread groundwater contaminants. Enrichment cultures XR_{DCA} and XR_{TCA} derived from river sediment dihaloeliminated 1,2-DCA to ethene and 1,1,2-TCA to vinyl chloride (VC), respectively. The XR_{TCA} culture subsequently converted VC to ethene via hydrogenolysis. Microbial community profiling demonstrated the enrichment of *Geobacter* 16S rRNA gene sequences in both the XR_{DCA} and XR_{TCA} cultures, and *Dehalococcoides mccartyi* (*Dhc*) sequences were only detected in the ethene-producing XR_{TCA} culture. The presence of a novel *Geobacter* population, designated as *Geobacter* sp. strain IAE, was identified by 16S rRNA gene-targeted polymerase chain reaction (PCR) and Sanger sequencing. Time-resolved population dynamics attributed the dihaloelimination activity to strain IAE, which attained growth yields of $0.93 \pm 0.06 \times 10^7$ and $1.18 \pm 0.14 \times 10^7$ cells per μmol Cl^{-1} released with 1,2-DCA and 1,1,2-TCA as electron acceptors, respectively. In contrast, *Dhc* growth only occurred during VC-to-ethene hydrogenolysis. Our findings discover a *Geobacter* sp. strain capable of respiring multiple chlorinated ethanes and demonstrate the involvement of a broader diversity of organohalide-respiring bacteria in the detoxification of 1,2-DCA and 1,1,2-TCA.

Synopsis

This work expands the range of chlorinated electron acceptors utilized by the genus *Geobacter*, with potential implications for improved remedial treatment of contaminated groundwater aquifers and sediments.

Keywords

Geobacter, chlorinated ethanes, dihaloelimination, 1,1,2-trichloroethane, 1,2-dichloroethane, organohalide respiration, bioremediation.

Introduction

Chlorinated ethanes such as 1,2-dichloroethane (1,2-DCA) and 1,1,2-trichloroethane (1,1,2-TCA) have been manufactured for a variety of industrial and agricultural applications, including solvents, degreasing agents, pesticides and chemical intermediates.[1] According to the U.S. Environmental Protection Agency (USEPA), the annual production volumes of 1,2-DCA and 1,1,2-TCA in the U.S. from 2012 to 2015 are estimated to range between 20,000-30,000 and 100-250 million pounds, respectively.[2, 3] Over 90% of globally produced 1,2-DCA is consumed by the polyvinyl chloride industry for the synthesis of vinyl chloride (VC) monomer.[4] The primary use of 1,1,2-TCA is for the production of 1,1-dichloroethene, a starting material for making plastic wrap and several industrial cleaning solvents.[5]

Improper storage, landfilling and accidental spillages caused widespread groundwater contamination with 1,2-DCA and 1,1,2-TCA, threatening ecosystem functions, clean water supply and human health.[6, 7] Toxicological studies demonstrated that human exposure to 1,2-DCA or 1,1,2-TCA can cause adverse effects to liver, kidney, immune system and central nervous system.[8] The USEPA has classified 1,2-DCA as a probable human carcinogen (Group 2B) and 1,1,2-TCA as a possible human carcinogen (Group 2C).[5, 9] In 2019, the USEPA further designated 1,2-DCA and 1,1,2-TCA as high priority substances for risk evaluation under the Toxic Substances Control Act (TSCA), a regulatory framework for the management and compliance of hazardous chemicals.[10, 11] The ongoing TSCA assessments rely on refined information of 1,2-DCA and 1,1,2-TCA including occupational health hazards, exposure pathways and environmental persistence and fate, of which the latter requires a comprehensive update on their biodegradability, transformation mechanisms and formation of toxic transformation products.

Organohalide respiration (OHR), the use of organohalogens as electron acceptors in energy-conserving reductive dehalogenation, is the main biotic process to attenuate 1,2-DCA and 1,1,2-TCA in oxygen-depleted aquifers and sediments.[12, 13] Dechlorination pathways of 1,2-

DCA and 1,1,2-TCA and the subsequent transformation of less chlorinated daughter products are illustrated in Figure 1. Dechlorination of 1,2-DCA can proceed via dihaloelimination to produce environmentally benign ethene.[14] Dihaloelimination of 1,1,2-TCA to VC followed by VC hydrogenolysis also leads to ethene formation.[15] Additionally, 1,2-DCA can be transformed via dehydrochlorination and transient VC accumulation has been observed in several *Dehalococcoides mccartyi* (*Dhc*) cultures.[16, 17] Since the oxidation state of carbon skeleton remains unchanged, dehydrochlorination is not a redox reaction and cannot be coupled with energy conservation and bacterial growth.[18]

The ability to metabolize 1,2-DCA and/or 1,1,2-TCA had been identified in a few groups of organohalide-respiring bacteria (OHRB) (Table S1). *Desulfitobacterium* sp. strain PR utilizes 1,1,2-TCA as electron acceptor and generates 1,2-DCA and chloroethane (CA) via sequential hydrogenolysis, albeit the 1,2-DCA-to-CA dechlorination process did not support OHR.[13,19] Dihaloelimination is more commonly observed in 1,2-DCA and/or 1,1,2-TCA-dechlorinating OHRB. For example, *Dhc* strain 195 and strain BAV1 grow on 1,2-DCA and generate predominantly ethene, but these *Dhc* strains show no activity towards 1,1,2-TCA.[16, 17] *Desulfitobacterium dichloroeliminans* strain LMG P-21439 was the first isolate demonstrated of dihaloeliminating 1,2-DCA and 1,1,2-TCA to ethene and VC, respectively.[20] Dihaloelimination of 1,2-DCA, 1,1,2-TCA and other vicinally chlorinated C2-C3 alkanes (e.g., 1,1,2,2-tetrachloroethane, 1,2-dichloropropane) has also been observed in several *Dehalogenimonas* (*Dhgm*) spp. isolates.[21, 22] *Dehalobacter restrictus* (*Dhb*) strains have been implicated in dihaloelimination of 1,2-DCA to ethene and 1,1,2-TCA to VC in mixed cultures.[8, 23, 24] Notably, based on the information disclosed in a patent,[25] *Geobacter* sp. strain AY is able to dechlorinate 1,2-DCA to ethene but cannot utilize 1,1,2-TCA.

Enhanced dihaloelimination of 1,1,2-TCA to VC was reported in wetland sediment microcosms during active ferric iron reduction.[26] Microcosms with robust ferric iron-reducing activity produced twice as much VC compared with their methanogenic counterparts.[26] The findings of a recent isotope fractionation study suggest that *Geobacter* was responsible for 1,2-DCA dechlorination at a chlorinated solvent-contaminated site in Italy.[27] High abundances of sequences affiliated with *Geobacter* were also detected in a 1,2-DCA-dihaloeliminating bioelectrochemical laboratory reactor[28] and at a 1,1,2-TCA-contaminated site in China.[6] These observations suggest that not yet identified members of the genus *Geobacter* contribute to the anaerobic degradation of chlorinated ethanes.

In this study, we obtained enrichment cultures from a freshwater sediment microbiome that completely dechlorinated 1,2-DCA and 1,1,2-TCA to ethene. Amplicon sequencing and targeted polymerase chain reaction (PCR) approaches identified that a novel *Geobacter* sp. strain dihaloeliminated 1,2-DCA and 1,1,2-TCA to ethene and VC, respectively. Complete detoxification of 1,1,2-TCA to ethene was achieved in the enrichment culture through synergistic cooperation between this dihaloeliminating *Geobacter* population and VC-dechlorinating *Dhc*.

Materials and Methods

Chemicals. 1,2-DCA and 1,1,2-TCA (both ≥ 97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethene, VC and methane (all ≥ 99.95%) were purchased from Dalian Special Gases (Dalian, Liaoning, China). All other chemicals were of analytical or higher grade.

Sediment sampling and enrichment culture setup. Freshwater sediment was sampled from the Xi River (41.6628°N, 123.1055°E, Shenyang, Liaoning, China) that is impacted by a variety of contaminants, including organohalogens and heavy metals.[29] Inside an anoxic chamber (Coy Laboratory Products, Ann Arbor, MI, USA), approximately 5 mL of homogenized sediment slurry was transferred to 120 mL glass serum bottles containing a N_2/CO_2 headspace (80/20, v/v) and 80 mL of bicarbonate buffered (30 mM, pH 7.2), reduced mineral salt medium[30] amended with 5 mM lactate and the Wolin vitamin mix[30] with 50 μg L^{-1} vitamin B_{12}. Electron donors (i.e., acetate and H_2) were generated from the fermentation of lactate as shown in eq 1.[31]

$$CH_3CHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2 \qquad (1)$$

The bottles were sealed with new, autoclaved black butyl rubber stoppers and crimped with aluminum caps. Neat 1,2-DCA (*ca*. 8 μL or 101.3 μmol, 1.2 mM aqueous concentration) or 1,1,2-TCA (*ca*. 8 μL or 83.5 μmol, 1.1 mM aqueous concentration) was added as an electron acceptor. The ratios of lactate to 1,2-DCA and 1,1,2-TCA in electron equivalents were estimated as 23.7 and 14.4, respectively (See the Supporting Information). Following complete conversion to ethene, repeated transfers (3%, v/v) to the same growth medium yielded solid-free 1,2-DCA- and 1,1,2-TCA-dechlorinating enrichment cultures designated as XR_{DCA} and XR_{TCA}, respectively. To track OHRB growth, triplicate XR_{TCA} cultures were prepared in 160 mL bottles containing 100 mL growth medium amended with 12 μL 1,1,2-TCA (*ca*. 125.2 μmol, 1.2 mM aqueous concentration). Autoclaved cultures amended with 1,2-DCA or 1,1,2-TCA served as negative controls. All bottles were incubated statically at 30°C in the dark with the stoppers facing up.

DNA extraction and 16S rRNA gene amplicon sequencing. Following complete dechlorination of 1,2-DCA or 1,1,2-TCA to ethene, cells were harvested from 1-mL culture suspensions via vacuum filtration onto 25-mm diameter 0.22-μm pore size membrane filters (Millipore, Burlington, MA, USA).[32] Genomic DNA was extracted from the filters using the TIANamp Soil DNA Kit (TIANGEN Inc., Beijing, China) following the manufacturer's protocol. DNA concentrations were determined using a Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). The V3-V4 regions of bacterial and archaeal 16S rRNA genes were amplified using primer set V3-V4-F and V3-V4-R (Table S2). The 25 μL assays contained 20 ng DNA template, 200 nM concentrations of each primer, 0.5 μL TransStart Taq, 2.5 μL TransStart buffer (TransGen Biotech, Beijing, China), 200 μM of each dNTP and nuclease-free water. The PCR cycle conditions were as follows: denaturation at 94°C for 3 min followed by 24 cycles of 94°C for 5 sec, annealing at 57°C for 90 sec, and extension at 72°C for 10 sec, and a final extension at 72°C for 5 min. Libraries were multiplexed by ligating adaptors containing different 6-bp index sequences to each fragmented DNA sample and were pooled at equimolar concentrations (i.e., 10 nM). Paired-end (2 x 250 bp) sequencing was performed using an Illumina MiSeq PE250 (Illumina Inc., San Diego, CA, USA) following established protocols.[33] Sequences with ambiguous nucleotides ('N') or smaller than 200-bp were filtered and removed using the Flash software version 1.2.11.[34] Following quality control, sequences were grouped into operational taxonomic units (OTUs) at a threshold of 97% sequence similarity using the SILVAngs server[35] with the default settings.

PCR and Sanger sequencing. Amplification of *Geobacter*, *Dhc*, and *Dhgm* 16S rRNA genes and the VC-dechlorinating reductive dehalogenase (RDase) genes *vcrA* and *bvcA* was performed on a Veriti™ 96-Well Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) using published primer sets (Table S2). The 50 *µ*L PCR reaction mixture contained 25 *µ*L of Premix Taq™ master mix (Takara Bio Inc., Beijing, China), 2 *µ*L DNA template, 0.2 *µ*M each primer, and nuclease-free water. Genomic DNA extracted from axenic cultures of *Dhc* strain BAV1 (ATCC BAA-2100),[17] *Dhc* strain GT (ATCC BAA-2099)[36] and '*Candidatus* Dehalogenimonas etheniformans' strain GP (JCM 39172)[37] were used as positive controls for *Dhc* 16S rRNA gene/*bvcA*, *vcrA* and *Dhgm* 16S rRNA gene specific PCR assays, respectively. The PCR cycle conditions were as follows: denaturation at 94 ^{o}C for 5 min, followed by 30 cycles of 98 ^{o}C for 10 s, annealing for 30 s, and extension at 72 ^{o}C for 1 min, and a final extension at 72°C for 10 min. The annealing temperature for each PCR assay is listed in Table S2. PCR products were separated using agarose gel (2%) electrophoresis, stained with GelRed and imaged using a Gel DOC™ XR+ imaging system (Bio-Rad Laboratories, Hercules, CA, USA). Sanger sequencing was performed using an Applied Biosystems 3730XL sequencer (Thermo Fisher Scientific) as described.[38] Sequencing chromatograms were manually inspected and assembled using Geneious version 11.1.2 (Biomatters Inc., Newark, NJ, USA).[39]

Phylogenetic analysis. Select *Geobacter*, *Dhc* and *Dhgm* 16S rRNA gene sequences were retrieved from GenBank and aligned using the MAFFT version 7.388 plug-in in Geneious version 11.1.2.[40] The phylogenetic tree was built based on partial 16S rRNA gene sequences using the neighbor-joining algorithm with Jukes-Cantor correction followed by 100 bootstrap analysis with PhyML in Geneious version 11.1.2.[41]

Quantitative PCR (qPCR). Primer set Geo16S_762qF (5'-GACGCTGAGACGCGAAAGC-3') and Geo16S_899qR (5'-TCCCAGGCGGAGTACTTAA-3') and TaqMan probe Geo16S_865P (5'-6FAM-CTGCAGTGCCGYAGCTAACGC-MGB-3') targeting the 16S rRNA gene of dechlorinating *Geobacter* population in enrichment cultures XR_{DCA} and XR_{TCA} were designed using the Primer3 plug-in in Geneious 11.1.2.[42] Assay specificity was verified by *in silico* analysis using Primer3 and the Primer-BLAST tool provided by NCBI.[42, 43] *Dhc* 16S rRNA gene copies were enumerated using published primer set Dhc1200F and Dhc1271R and TaqMan probe Dhc1240probe (Table S2). The qPCR assays were performed on a QuantStudio™ 3 Real-Time PCR system (Thermo Fisher Scientific). Each 25 μL assay contained 12.5 μL 2 x Premix Ex Taq™ master mix (Takara Bio Inc.), 200 nM of each primer and probe, 0.25 μL 50 x ROX reference Dye II (Takara Bio Inc.), 2 μL DNA template and nuclease-free water. The cycle conditions were as follows: 95 ^{o}C for 30 s, followed by 40 cycles of 5 s at 95 °C and 34 s at 60 °C. Calibration curves were generated using three independent dilution series of plasmid DNA carrying a fragment of *Geobacter lovleyi* strain SZ 16S rRNA gene (GenBank accession number AY914177.1) or a *Dhc* 16S rRNA gene (GenBank accession number AY165308.1). The *Geobacter* qPCR assay had an amplification efficiency of 80%, a detection limit of 1.10 x 10^2 gene copies per reaction tube, and a linear dynamic range spanning 1.10 x 10^2 to 1.10 x 10^9 gene copies per reaction tube (Figure S1). Cell number calculations assumed two 16S rRNA gene copies per genome for *Geobacter*[44] and one copy for *Dhc*.[45]

Analytical methods. Methane, ethene and chlorinated compounds were analyzed using an Agilent 7697A GC headspace autosampler (Agilent Technologies, Santa Clara, CA, USA) connected to an Agilent 7890B gas chromatograph equipped with an Agilent DB-624 column (60 m length x 0.32 mm diameter, 1.8 μm film thickness) and a flame ionization detector.[46] The method detection limits were ~0.2 μM for 1,2-DCA and 1,1,2-TCA, ~0.3 μM for VC and ~1 μM for ethene. Dechlorination rates were estimated based on the changes of nominal concentration of 1,2-DCA, 1,1,2-TCA or VC over a period of linear decrease represented by at least three measurements. Growth yields were calculated as (1) the increase in biomass per mol of chlorinated substrate consumed and (2) the increase in cell numbers divided by the total amount of chloride ions released during the consumption of chlorinated electron acceptors.[47] The dry weights of OHRB cells were estimated in Table S3. Dihaloelimination releases two chloride ions per 1,2-DCA or 1,1,2-TCA molecule and hydrogenolysis releases one chloride ion per VC molecule.

Data availability. The amplicon sequencing data are available in the Sequence Read Archive of GenBank under the accession number SUB9283928. The BioProject accession number is PRJNA635757. The partial 16S rRNA gene sequence of *Geobacter* sp. strain IAE is available under the accession number MW990083.

Results

River sediment microbiome dechlorinates 1,2-DCA and 1,1,2-TCA to ethene. The Xi River sediment microcosms dechlorinated 87.8 ± 2.8 μmol 1,2-DCA to stoichiometric amounts of ethene within 14 days (Figure 2A). After five consecutive transfers, the XR_{DCA} culture maintained the ability to dechlorinate 1,2-DCA to ethene at an apparently increased rate of 319.3 ± 45.9 μM day^{-1} (Figure 2B). VC, a potential intermediate of 1,2-DCA dechlorination, was never detected (i.e., below detection limit) in the XR_{DCA} transfer cultures, suggesting a dihaloelimination reaction directly leading to ethene formation.

Ethene was also observed as the dechlorination end product in the 1,1,2-TCA amended sediment microcosms. The measured amounts of 64.9 ± 2.8 μmol 1,1,2-TCA at the beginning of incubation was 19-25% less than the expected value (83.5 μmol), indicating some 1,1,2-TCA was sorbed to the butyl rubber stoppers. The initial 1,1,2-TCA was completely dihaloeliminated to VC within 14 days and a maximum amount of 53.8 ± 0.8 μmol VC was measured before further hydrogenolysis to ethene occurred (Figure 2C). The second transfer XR_{TCA} culture exhibited the same dechlorination pattern and dihaloelimination of 1,1,2-TCA to VC was followed by VC hydrogenolysis to ethene during a 28-day incubation period (Figure 2D). In negative control incubations, neither a dechlorination product (e.g., a dichloroethene, VC, ethene) nor significant changes in the total masses of 1,2-DCA or 1,1,2-TCA was observed (Figure S2). At the end of incubation, the sediment microcosms had accumulated 50-80 μmol methane bottle^{-1} and similar amounts were measured in the XR_{DCA} (60-70 μmol bottle^{-1}) and XR_{TCA} (70-80 μmol bottle^{-1}) cultures, indicating active methanogenesis. Taken together, these results demonstrate that Xi River sediment harbors microbes that dechlorinate 1,2-DCA and 1,1,2-TCA to ethene.

Community structure of sediment microcosms and enrichment cultures. A total of 187,999 high-quality sequences assigned to 10,254 OTUs were obtained from the sediment microcosms and enrichment cultures. The rarefaction plots did not plateau (Figure S3), indicating that the amplicon sequencing results may underestimate the true species richness.

As shown in Table S4, *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Chloroflexi* were the dominant phyla accounting for 66.7-79.5% of the total sequences in the respective sediment microcosm that completely dechlorinated 1,2-DCA or 1,1,2-TCA to ethene. While *Proteobacteria* (22.1-36.6%), *Firmicutes* (24.9-27.6%) and *Bacteroidetes* (24.1-30.3%) remained the most abundant phyla in the enrichment cultures, the relative abundances of *Chloroflexi* sequences decreased from 6.8-8.0% in the microcosms to 0.05% and 2.38% in the XR_{DCA} and XR_{TCA} cultures, respectively. *Euryarchaeota* was the most abundant archaeal phylum in the sediment microcosms (3.2-5.1%) and enrichment cultures (3.7-6.2%). *Crenarchaeota* was the only other archaeal phylum present at ≥ 0.01% abundance in the sediment microcosms (0.03-0.04%) but it was not detected in the enrichment cultures. Other phyla detected at abundances of >2% included *Cloacimonetes* (2.6-5.0%) and *Caldiserica* (3.3-3.6%) in the sediment microcosms, and *Cloacimonetes* (3.1-4.0%) and *Spirochaetes* (2.3-3.4%) in the enrichment cultures. Of note, sequences affiliated with the WPS-2 cluster,[48] an unclassified phylum related to the *Chloroflexi*,[49] increased from 0.01% in the sediment microcosms to 0.3-1.0% relative abundances in the enrichment cultures.

At the genus level (Figure 3 and Table S5), the most abundant OTUs in the sediment microcosms belong to the unclassified environmental group "vadinH17" (21.5-23.9%) within the *Bacteroidetes* phylum;[50] however, "vadinH17" sequences were either absent or detected at relative abundances below 0.4% in the enrichment cultures. As shown in Table 1, *Geobacter* OTUs contributed 1.1% and 6.0% of the total sequences in the 1,2-DCA- and 1,1,2-TCA-dechlorinating sediment microcosms, respectively. Following six consecutive transfers, *Geobacter* sequences dominated the sequence pool in the XR_{DCA} culture and accounted for 34.5% of the total sequences. Similarly, the sequences affiliated with *Geobacter* OTUs increased to 13.4% in the XR_{TCA} culture. The relative abundances of *Dhc* OTUs, which represented 0.35-0.51% of the total sequences in the sediment microcosms, increased to 1.8% in the XR_{TCA} culture but no *Dhc* sequences were detected in the XR_{DCA} culture. The majority of archaeal sequences were affiliated with *Methanocorpusculum*, which was present at abundances of 2.2-3.6% in the sediment microcosms and 3.7-5.9% in the enrichment cultures (Table S5). *Methanosaeta* (0.29-0.34%) and *Methanosarcina* (0.06-0.36%) sequences were present in the sediment microcosms but were not detected in the enrichment cultures (Table S5). *Methanofollis* sequences, which represented a minor fraction (0.01-0.02%) of the total sequences in the sediment microcosms, increased to 0.12% in the XR_{TCA} culture but were undetected in the XR_{DCA} culture (Table S5).

Other than *Geobacter*, *Dhb* was the only OHR phylotype observed in both the XR_{DCA} and XR_{TCA} cultures, albeit at extremely low abundances accounting for no more than 0.03% of the total sequences (Table 1). Known 1,2-DCA- and/or 1,1,2-TCA-dechlorinating phylotypes including *Desulfitobacterium* and *Dhgm* were either never detected or initially present at low abundances in the sediment microcosms but vanished after repeated transfers (Table 1).

Overall, unexpected high abundances of *Geobacter* sequences in the enrichment cultures strongly suggested the involvement of *Geobacter* in the dihaloelimination of 1,2-DCA and 1,1,2-TCA.

Identification of a novel *Geobacter* sp. strain implicated in 1,2-DCA and 1,1,2-TCA dechlorination. To reveal the identity of the *Geobacter* population(s) implicated in the observed dechlorination activities, amplicons containing a partial, ~850 bp-long 16S rRNA gene sequence using *Geobacter*-specific primers were obtained from the XR_{DCA} and XR_{TCA} cultures (Figure S4). The obtained amplicon sequences were 100% identical to each other, suggesting that the same *Geobacter* sp. strain was enriched with 1,2-DCA or 1,1,2-TCA as the electron acceptor. Pairwise comparisons of 16S rRNA gene sequences corroborated this strain's affiliation with the genus *Geobacter* (92.3-99.0% sequence similarities). As shown in Figure 4, the highest 16S rRNA gene sequence similarities of 98.9-99.0% exist with the tetrachloroethene- (PCE-) dechlorinating species *Geobacter lovleyi*,[51, 52] which presumably also accommodates the 1,2-DCA-dechlorinating *Geobacter* sp. strain AY.[25]

The ability to dechlorinate VC to ethene is limited to *Dhc* and *Dhgm* strains carrying a VC RDase gene (e.g., *bvcA*,[53] *vcrA*,[54] *tceA*,[46] *cerA*[37]). To explore the presence of known VC dechlorinators, we performed PCR assays targeting *Dhc* and *Dhgm* biomarker genes in the enrichment cultures. Consistent with the community profiles, *Dhc* 16S rRNA gene amplicon was detected using template DNA from the XR_{TCA} culture but no amplicon was observed using template DNA from the XR_{DCA} culture (Figure S4). The detection of *vcrA*- and *bvcA*-specific amplicons from the XR_{TCA} culture provide additional evidence that one or more *Dhc* strains in the XR_{TCA} culture possess genes implicated in VC hydrogenolysis (Figure S4). Attempts to amplify *Dhgm* 16S rRNA genes were unsuccessful from both the XR_{DCA} and the XR_{TCA} cultures (Figure S4). Taken together, the PCR and Sanger sequencing results revealed the presence of a novel *Geobacter* sp. strain, designated as strain IAE (for Institute of Applied Ecology, the location where this work was performed), involved in 1,2-DCA and 1,1,2-TCA dihaloelimination in enrichment cultures XR_{DCA} and XR_{TCA}, respectively.

Synergistic roles of *Geobacter* and *Dhc* in 1,1,2-TCA detoxification. Following the consumption of the initial amount of 94.4 ± 1.6 μmol 1,2-DCA, strain IAE cell numbers increased by 48.2-fold from the 3.73 ± 0.83 x 10^5 (i.e., cells introduced with the inoculum) to 1.80 ± 0.10 x 10^7 cells mL^{-1} by the end of incubation period (Table 2). We next tracked the growth of *Geobacter* and *Dhc* in the XR_{TCA} culture at different dechlorination stages (i.e., dihaloelimination of 1,1,2-TCA followed by hydrogenolysis of VC). Concomitant with 1,1,2-TCA dihaloelimination to VC at rates of 142.8 ± 6.9 μM day^{-1}, strain IAE cell densities increased from the initial 5.15 ± 0.23 x 10^5 to 2.86 ± 0.50 x 10^7 cells mL^{-1} at day 10 (Table 2) when a maximum amount of VC (98.4 ± 10.1 μmol) had accumulated (Figure 5). No further increases in strain IAE cell numbers were measured during subsequent VC-to-ethene hydrogenolysis, which occurred at rates of 50.1 ± 8.9 μM day^{-1} from day 10 to day 37 (Figure 5), indicating that strain IAE cannot utilize VC as an energy substrate. Similar growth yields of 0.93 ± 0.06 x 10^7 and 1.18 ± 0.14 x 10^7 cells per μmol Cl^{-1} released were calculated for strain IAE using 1,2-DCA and 1,1,2-TCA as the electron acceptors, respectively (Table 2), reflecting the changes in Gibbs free energy ($\Delta G^{o\prime}$) associated with the dihaloelimination reactions of 1,2-DCA-to-ethene (-85.27 KJ mol^{-1} [acetate as electron donor] or -121.35 KJ mol^{-1} [H_2 as electron donor]) and 1,1,2-TCA-to-VC (-97.28 KJ mol^{-1} [acetate as electron donor] or -133.36 KJ mol^{-1} [H_2 as electron donor]) (Table S6). These growth yields are in the same range reported for other OHRB (e.g., *Dhgm*, *Dhb*) able to dechlorinate 1,2-DCA and/or 1,1,2-TCA. For comparison, the growth yields of OHRB that can grow with 1,2-DCA and/or 1,1,2-TCA and *Geobacter lovleyi* strains are provided in Table S7. In cultures that did not receive 1,2-DCA or 1,1,2-TCA amendment, negligible increases of strain IAE cell numbers were measured (Table 2).

In contrast, very limited *Dhc* growth occurred during the stage of 1,1,2-TCA-to-VC dihaloelimination and the *Dhc* cell numbers merely increased from 1.85 ± 0.28 x 10^6 (cells introduced with the inoculum) to 2.47 ± 0.17 x 10^6 cells mL^{-1} at day 10 (Figure 5 and Table 2). Significant increases in *Dhc* cell densities were observed concomitantly with ethene formation. Following complete VC hydrogenolysis to ethene, *Dhc* cell densities increased by 28.0-fold from 2.47 ± 0.17 x 10^6 to 6.92 ± 0.91 x 10^7 cells mL^{-1} (Figure 5 and Table 2). The calculated growth yield of 7.59 ± 0.84 x 10^7 cells per μmol ethene formed is on par with yield values reported for VC-dechlorinating *Dhc* isolates.[45]

About double the amount of *Dhc* cells were produced compared to strain IAE cells in the XR_{TCA} culture (Table 2), albeit VC-to-ethene hydrogenolysis yields slightly less free energy ($\Delta G^{o\prime}$ = -114.51 kJ mol^{-1}) than 1,1,2-TCA-to-VC dihaloelimination ($\Delta G^{o\prime}$ = -133.36 KJ mol^{-1}) with hydrogen as electron donor (Table S6). In terms of biomass yield, XR_{TCA} culture produced 1.10 ± 0.19 g of strain IAE cells per mol of 1,1,2-TCA consumed and 1.38 ± 0.15 g of *Dhc* cells per mol of VC consumed (Table 2). These values are more consistent with the free energy changes associated with the respective dechlorination reactions, suggesting that yields based on biomass (i.e., dry weight) should be used for comparison of growth yields of bacteria with considerably different cellular dimensions.[55]

The qPCR analysis delineated the distinct roles of *Geobacter* and *Dhc* in the XR_{TCA} culture. These results demonstrate that strain IAE couples growth with dihaloelimination of 1,2-DCA and 1,1,2-TCA, an OHR capacity that has not been previously associated with the genus *Geobacter*.

Discussion

Elucidating the microbiology controlling the transformation pathways and formation of toxic intermediates can provide a better understanding of the environmental fate and longevity of chlorinated ethanes (e.g., 1,2-DCA, 1,1,2-TCA) at contaminated sites. Recent studies reported elevated abundances of sequences affiliated with the *Geobacter* genus in systems with 1,2-DCA- or 1,1,2-TCA-dechlorination activity;[6, 27] however, definitive roles for *Geobacter* in these studies were not assigned. Our results provide explicit evidences that strain IAE represents a novel OHRB in the genus *Geobacter* capable of dechlorinating 1,2-DCA and 1,1,2-TCA to ethene and VC, respectively. The absence or marginal presence of known 1,2-DCA or 1,1,2-TCA dechlorinators (e.g., *Dhb, Dhgm*) indicates that strain IAE is the major contributor to the observed dihaloelimination activities. The conclusion that strain IAE is a dihaloeliminating OHRB is further supported by tracking its growth over the course of 1,2-DCA and 1,1,2-TCA dechlorination to ethene. Our new findings reveal that a broader OHRB diversity utilizes chlorinated ethanes as growth-supporting electron acceptors.

Expanded OHR capacity in *Geobacter*. Members within the genus *Geobacter* are metabolically versatile and broadly distributed in subsurface environments such as sediments and groundwater aquifers.[56] A hallmark feature of *Geobacter* spp. is the ability to reduce oxidized metals and perform extracellular electron transfer to insoluble electron acceptors.[57] To date, the capability to utilize chlorinated electron acceptors have been well characterized in *Geobacter lovleyi* strains.[51, 52] *Geobacter lovleyi* strain SZ and the recently isolated strain LYY reduce PCE and trichloroethene (TCE) to *cis*-1,2-dichloroethene (*c*DCE) via hydrogenolysis.[51, 52] *Geobacter lovleyi* strain SZ cannot dechlorinate 1,2-DCA and 1,1,2-TCA[51] and strain LYY's ability to dechlorinate 1,2-DCA or 1,1,2-TCA has not been determined.[52] *Geobacter* sp. strain AY, for which information in a peer-reviewed literature is lacking, was claimed to only dechlorinate 1,2-DCA but not 1,1,2-TCA.[25] *Geobacter thiogenes* strain K1, a close relative of *Geobacter lovleyi*, converts trichloroacetate to dichloroacetate[58]; however, growth requires sulfur-mediated electron shuttling suggesting this bacterium is not an OHRB *sensu stricto*.[59]

The observation that a *Geobacter* sp. strain able to metabolize 1,2-DCA and 1,1,2-TCA explains prior studies showing that 1,1,2-TCA dihaloelimination to VC was stimulated in sediment microcosms with ferric iron-reducing activity.[26] OHR and iron reduction are not exclusive processes and *Geobacter lovleyi* strain SZ simultaneously reduces PCE and ferric iron.[51] Concomitant consumption of ferric iron and a chlorinated electron acceptor (i.e., 2-chlorophenol) was also observed in *Anaeromyxobacter dehalogenans* strain 2CP-2 cultures,[60] suggesting that OHR and iron reduction can occur simultaneously.

Based on genome-wide analyses, the taxonomy of the class *Deltaproteobacteria* has been revised recently along with a controversial proposal for nomenclature changes.[61, 62] Without genome information, a taxonomic assignment at the species level for strain IAE cannot be faithfully determined; however, 16S rRNA gene phylogeny and *Geobacter lovleyi* genome information[63] support that organohalide-respiring members of the family *Geobacteraceae* form a distinct subclade and group with the species *Geobacter lovleyi*.[62]

Dihaloelimination is preferred for 1,2-DCA and 1,1,2-TCA detoxification. A common issue at many National Priorities List (NPL) sites is incomplete dechlorination, which often results in less chlorinated but potentially more toxic compounds.[64] Of particular health concern is the formation of VC, a human carcinogen (Group A), for which a maximum concentration level of 2 ppb in groundwater is generally enforced.[65] Besides generating VC via dehydrochlorination, 1,2-DCA also can undergo biotically-mediated hydrogenolysis to produce chloroethane (CA) (Figure 1), which has been found in at least 131 of 1,327 active NPL sites[66] and is a hazardous air pollutant.[67] So far, biodegradation of CA via anaerobic processes including OHR has not been observed,[68, 69] suggesting that CA is persistent in anoxic environments. Therefore, stimulation and/or augmentation of dihaloeliminating OHRB (e.g., strain IAE) could achieve detoxification and avoid the formation of VC and CA at 1,2-DCA-contaminated sites.

The coexistence of strain IAE and *Dhc* in the XR_{TCA} culture achieves complete dechlorination of 1,1,2-TCA to ethene, a detoxification process that is accomplished by two distinct OHRB exhibiting complementary dechlorination activities. In a *Dhgm*-containing culture augmented with *Dhc*, the *Dhgm* population performed dihaloelimination and transformed 1,1,2-TCA to VC, which was further reduced to ethene by *Dhc*.[15] Similarly, a *Dhb* population capable of dihaloeliminating 1,1,2-TCA to VC was identified in a mixed culture and the generated VC was dechlorinated to ethene by *Dhc*.[8] A co-culture constructed with *Desulfitobacterium* sp. strain PR and *Dhc* strain 11a also completely dechlorinated 1,1,2-TCA to ethene. Strain PR catalyzed the hydrogenolysis of 1,1,2-TCA to 1,2-DCA, and strain 11a dihaloeliminated 1,2-DCA to ethene, albeit TCE was required to suppress undesirable CA formation.[13] In any case, these examples illustrate that a dihaloeliminating OHRB is required for achieving complete detoxification of 1,1,2-TCA.

Synergistic interactions between *Geobacter* and *Dhc* achieve detoxification. Co-growth of *Geobacter* and *Dhc* to accomplish PCE-to-ethene dechlorination has been observed in the KB-1® consortium,[70] where *Geobacter* sp. strain KB-1 generated *c*DCE and *Dhc* converted *c*DCE to ethene. Here, we demonstrated that *Geobacter* sp. strain IAE in concert with one or more VC-dechlorinating *Dhc* strains achieve complete detoxification of 1,1,2-TCA to ethene. Dihaloelimination of 1,1,2-TCA by *Geobacter* provides a chlorinated electron acceptor, VC, and enables *Dhc* growth. In addition, *Geobacter* can supply essential compounds to meet *Dhc*'s nutritional requirements. For example, *Dhc* strains are corrinoid auxotrophs and rely on exogenous supply for growth.[71] In co-cultivation studies with *Dhc* strain BAV1, *Geobacter lovleyi* strain SZ dechlorinated PCE to *c*DCE and released cobamide, which complemented strain BAV1's nutritional requirement and enabled further detoxification of *c*DCE to ethene.[72] A potential benefit that *Dhc* offers to *Geobacter* is the consumption of hydrogen to enable syntrophic oxidation processes. *Dhc* strains are strictly hydrogenotrophic and can perform OHR at very low hydrogen concentrations.[73, 74] In a defined co-culture, *Geobacter lovleyi* strain LYY oxidized propionate to acetate and hydrogen, the latter two products serving as carbon source and electron donor, respectively, for *Dhc* strain CG1 to grow with polychlorinated biphenyls as an electron acceptor.[52] In return, the consumption of hydrogen by *Dhc* maintained favorable energetic conditions enabling propionate oxidation by strain LYY. Similarly, syntrophic acetate oxidation also requires a hydrogen-oxidizing partner population to maintain low hydrogen partial pressure.[75] This concept has been demonstrated in co-cultures of the non-dechlorinating *Geobacter sulfurreducens* and the nitrate-ammonifying bacterium *Wolinella succinogenes*, where the latter organism consumed hydrogen for dissimilatory nitrate reduction to ammonia and lowered the hydrogen partial pressure allowing *Geobacter sulfurreducens* to couple growth with acetate oxidation.[76]

The absence of *Dhc* in the XR_{DCA} culture provided evidence that strain IAE outcompeted *Dhc* during the utilization of a common substrate (i.e., 1,2-DCA); however, possibility that the indigenous *Dhc* in the Xi River sediment was unable to grow with 1,2-DCA cannot be excluded. In contrast, the sequential dechlorination of 1,1,2-TCA to ethene established different niches for strain IAE and *Dhc* and make these two populations not direct competitors, which may explain their abundant presences in the XR_{TCA} culture. Future studies should be conducted in defined systems (i.e., axenic cultures, co-cultures) to reveal whether a specific or a previously reported mechanism (e.g., a nutrient) enables the synergistic interactions between *Dhc* and this dihaloeliminating *Geobacter*.

Implications for bioremediation applications. In recent years, OHR-based bioremediation has emerged as a cornerstone process for the restoration of many contaminated sites impacted by anthropogenic chlorinated solvents.[18] The practical value of *Geobacter* for *in situ* bioremediation is exemplified in the commercial KB-1® consortium,[77] where *Geobacter* sp. strain KB-1 is stably maintained as the dominant PCE and TCE dechlorinator. The successful application of consortium KB-1® for *in situ* bioremediation[78] illustrates that organohalide-respiring *Geobacter* are robust dechlorinators and highlights the potentials for developing microbial consortia with expanded capabilities for efficient detoxification of chlorinated ethanes. Future studies will attempt the isolation of *Geobacter* sp. strain IAE to allow detailed physiological studies and the identification of biomarker gene(s) for monitoring dihaloelimination activity.

Notes

The authors declare no competing financial interests.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Table S1: Summary of known OHRB capable of dechlorinating 1,2-DCA and/or 1,1,2-TCA. Table S2: Nucleotide sequences of the primers and probes used in this study. Table S3: Estimation of the dry weight of different OHRB cells. Table S4: Phylum-level abundances based on 16S rRNA gene amplicon sequences obtained from the sediment microcosms and enrichment cultures. Table S5: Genus-level abundances based on 16S rRNA gene amplicon sequences obtained from the sediment microcosms and enrichment cultures. Table S6: Gibbs standard-state free energy changes of select dihaloelimination and hydrogenolysis reactions. Table S7: Growth yields of tetrachloroethene (PCE)-dechlorinating *Geobacter lovleyi* strains and select OHRB that utilize 1,2-DCA and/or 1,1,2-TCA as the electron acceptor. Figure S1: The amplification plot and standard curve of the qPCR assay targeting the *Geobacter* sp. strain IAE 16S rRNA gene. Figure S2: Stability of 1,2-DCA and 1,1,2-TCA in heat-killed negative control incubations. Figure S3: Rarefaction curves of 16S rRNA gene amplicon sequences recovered from sediment microcosm and enrichment culture samples. Figure S4: Gel electrophoresis of PCR amplicons generated using template DNA extracted from a XR_{DCA} or XR_{TCA} culture.

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Figure legends

Figure 1. Dechlorination pathways and OHRB phylotypes involved in the biotransformation of 1,2-DCA and 1,1,2-TCA under anoxic conditions. Hydrogenolysis is a reductive dechlorination reaction that replaces a chlorine atom with a hydrogen. Dihaloelimination is a reductive dechlorination that removes two adjacent chlorine atoms with the simultaneous formation of a carbon-carbon double bond. Dehydrochlorination is not a redox reaction and eliminates a chlorine atom and a hydrogen atom on adjacent C atoms with the simultaneous formation of a carbon-carbon double bond.

Figure 2. Reductive dechlorination of 1,2-DCA or 1,1,2-TCA in Xi River sediment microcosms and enrichment cultures. Panel A and panel B show the dihaloelimination of 1,2-DCA to ethene in sediment microcosm and the fifth transfer of the XR_{DCA} culture, respectively. Panel C and panel D show the complete dechlorination of 1,1,2-TCA to ethene in sediment microcosm and the second transfer of the XR_{TCA} culture, respectively. The error bars represent the standard deviations of triplicate samples and are not shown when smaller than the symbol.

Figure 3. Bacterial community structures at the phylum level (A) and the genus level (B) in the sediment microcosms and the XR_{DCA} (sixth transfer) and XR_{TCA} (second transfer) cultures following complete consumption of 1,2-DCA or 1,1,2-TCA, respectively. “Others” refers to OTUs affiliated with low-abundance phyla and genera (≤ 0.01% of total sequences).

Figure 4. Phylogenetic affiliation of strain IAE to select *Geobacter* spp. strains based on partial (825 bp) 16S rRNA gene sequences. Included as outgroups are *Dhc* and *Dhgm* 16S rRNA gene sequences. GenBank accession numbers are provided in parentheses. Bootstrap values expressed as a percentage of 1,000 resamplings are shown at the branching points. The scale bar indicates nucleotides substitutions per site.

Figure 5. Reductive dechlorination of 1,1,2-TCA to ethene (A) and growth of *Geobacter* sp. strain IAE and a *Dhc* population (B) in the XR_{TCA} culture. The two vertical lines above the top panel indicate the stages of dihaloelimination of 1,1,2-TCA to VC and hydrolysis of VC to ethene, respectively. The green frame box indicates the increase in *Geobacter* abundances in the enrichment cultures. The error bars represent the standard deviations of triplicate samples and are not shown when smaller than the symbol.

Table 1. The relative abundances of *Geobacter* and known 1,2-DCA and/or 1,1,2-TCA dechlorinating OHRB phylotypes at the genus level in sediment microcosms and enrichment cultures.

OHRB Phylotype	**Relative abundance (%)** [a]			
	1,2-DCA-dechlorinating		1,1,2-TCA-dechlorinating	
	Microcosm	XR_{DCA} [b]	Microcosm	XR_{TCA} [c]
Geobacter	6.0	**34.5**	1.1	**13.4**
Dhc	0.4	0	0.5	**1.8**
Dhb	0.7	0.03	0.8	0.03
Dsf [d]	0	0	0	0
Dhgm	0.02	0	0.05	0

[a] The relative abundance of each OHRB phylotype was estimated based on the percentage of its 16S rRNA gene sequences in the total sequenced amplicons. [b] DNA sample was extracted from the sixth transfer of a XR_{DCA} culture. [c] DNA sample was extracted from the second transfer of a XR_{TCA} culture. [d] *Dsf* abbreviates *Desulfitobacterium*.

Table 2. Growth of *Geobacter* sp. strain IAE and *Dhc* in enrichment cultures amended with 1,2-DCA or 1,1,2-TCA.

OHRB	Enrichment culture: dechlorination process	Cell densities (cells mL^{-1}) [a]		Fold increase [c]	Growth yield [d]	Biomass yield [e]
		Initial	Final [b]			
Strain IAE	XR_{DCA}: 1,2-DCA [f] → ethene	$3.73 \pm 0.83 \times 10^5$	$1.80 \pm 0.10 \times 10^7$	48.2	$0.93 \pm 0.06 \times 10^7$	0.87 ± 0.05
	XR_{TCA}: 1,1,2-TCA [g] → VC	$5.15 \pm 0.23 \times 10^5$	$2.86 \pm 0.50 \times 10^7$	55.4	$1.18 \pm 0.14 \times 10^7$	1.10 ± 0.19
	XR_{TCA}: VC [h] → ethene	$2.86 \pm 0.50 \times 10^7$	$2.44 \pm 0.45 \times 10^7$	0.8	- [i]	- [i]
	XR_{TCA}: - [j]	$1.07 \pm 0.26 \times 10^6$	$1.58 \pm 0.16 \times 10^6$	1.5	- [i]	- [i]
Dhc	XR_{TCA}: 1,1,2-TCA [g] → VC	$1.85 \pm 0.28 \times 10^6$	$2.47 \pm 0.17 \times 10^6$	1.3	- [i]	- [i]
	XR_{TCA}: VC [h] → ethene	$2.47 \pm 0.17 \times 10^6$	$6.92 \pm 0.91 \times 10^7$	28.0	$7.59 \pm 0.84 \times 10^7$	1.38 ± 0.15

[a] Average and the standard deviations of cell numbers were calculated from the measurements in triplicate cultures. [b] When a utilizable electron acceptor was completely depleted. [c] Fold increase in cell densities is reported as average of the measurements in triplicate cultures. [d] Growth yields were calculated as increases of cells per μmol Cl^- released. [e] Biomass yields were expressed as the total dry weight (gram) of increased cells per mol chlorinated substrate consumed. The dry weight of individual *Geobacter* and *Dhc* cells were estimated in Table S3. [f] 94.4 ± 1.6 μmol 1,2-DCA was consumed in this process. [g] 118.0 ± 6.6 μmol 1,1,2-TCA was consumed in this process. [h] 98.4 ± 10.1 μmol VC was consumed in this process. [i] Yields were not calculated for insignificant growth. [j] A chlorinated compound was not provided.