UCLA

UCLA Previously Published Works

Title

Dehalogenation of Chlorinated Ethenes to Ethene by a Novel Isolate, Candidatus Dehalogenimonas etheniformans.

Permalink

https://escholarship.org/uc/item/18z7q77p

Journal

Applied and Environmental Microbiology, 88(12)

Authors

Chen, Gao

Kara Murdoch, Fadime

Xie, Yongchao

et al.

Publication Date

2022-06-28

DOI

10.1128/aem.00443-22

Peer reviewed

Dehalogenation of Chlorinated Ethenes to Ethene by a Novel Isolate, "*Candidatus* Dehalogenimonas etheniformans"

Gao Chen,[a,b] Fadime Kara Murdoch,[a]* Yongchao Xie,[a,b]§ Robert W. Murdoch,[a]◇ Yiru Cui,[f,g] Yi Yang,[f] Jun Yan,[f] Trent A. Key,[h] Frank E. Löffler[a,b,c,d,e]

aCenter for Environmental Biotechnology, University of Tennessee, Knoxville, Tennessee, USA
bDepartment of Civil and Environmental Engineering, University of Tennessee, Knoxville, Tennessee, USA
cDepartment of Microbiology, University of Tennessee, Knoxville, Tennessee, USA
dDepartment of Biosystems Engineering and Soil Science, University of Tennessee, Knoxville, Tennessee, USA
eBiosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA
fKey Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, Liaoning, China
gUniversity of Chinese Academy of Sciences, Beijing, China
hExxonMobil Environmental and Property Solutions Company, Spring, Texas, USA

ABSTRACT *Dehalococcoides mccartyi* strains harboring vinyl chloride (VC) reductive dehalogenase (RDase) genes are keystone bacteria for VC detoxification in groundwater aquifers, and bioremediation monitoring regimens focus on *D. mccartyi* biomarkers. We isolated a novel anaerobic bacterium, "*Candidatus* Dehalogenimonas etheniformans" strain GP, capable of respiratory dechlorination of VC to ethene. This bacterium couples formate and hydrogen (H_2) oxidation to the reduction of trichloroethene (TCE), all dichloroethene (DCE) isomers, and VC with acetate as the carbon source. Cultures that received formate and H_2 consumed the two electron donors concomitantly at similar rates. A 16S rRNA gene-targeted quantitative PCR (qPCR) assay measured growth yields of $(1.2 \pm 0.2) \times 10^8$ and $(1.9 \pm 0.2) \times 10^8$ cells per μmol of VC dechlorinated in cultures with H_2 or formate as electron donor, respectively. About 1.5-fold higher cell numbers were measured with qPCR targeting *cerA*, a single-copy gene encoding a putative VC RDase. A VC dechlorination rate of 215 ± 40 μmol L^{-1} day^{-1} was measured at 30°C, with about 25% of this activity occurring at 15°C. Increasing NaCl concentrations progressively impacted VC dechlorination rates, and dechlorination ceased at 15 g NaCl L^{-1}. During growth with TCE, all DCE isomers were intermediates. Tetrachloroethene was not dechlorinated and inhibited dechlorination of other chlorinated ethenes. Carbon monoxide formed and accumulated as a metabolic by-product in dechlorinating cultures and impacted reductive dechlorination activity. The isolation of a new *Dehalogenimonas* species able to effectively dechlorinate toxic chlorinated ethenes to benign ethene expands our understanding of the reductive dechlorination process, with implications for bioremediation and environmental monitoring.

IMPORTANCE Chlorinated ethenes are risk drivers at many contaminated sites, and current bioremediation efforts focus on organohalide-respiring *Dehalococcoides mccartyi* strains to achieve detoxification. We isolated and characterized the first non-*Dehalococcoides* bacterium, "*Candidatus* Dehalogenimonas etheniformans" strain GP, capable of metabolic reductive dechlorination of TCE, all DCE isomers, and VC to environmentally benign ethene. In addition to hydrogen, the new isolate utilizes formate as electron donor for reductive dechlorination, providing opportunities for more effective electron donor delivery to the contaminated subsurface. The discovery that a broader microbial diversity can achieve detoxification of toxic chlorinated ethenes in anoxic aquifers illustrates the potential of naturally occurring microbes for biotechnological applications.

Drinking water aquifers are under increasing threat from excessive exploitation (e.g., withdrawal for irrigation), insufficient aquifer recharge due to changing precipitation patterns, and anthropogenic pollutants that impact water quality. Legacy chlorinated solvent contamination poses a lingering problem in many countries (1, 2). Chlorinated solvents, foremost chlorinated ethenes, are known or probable human carcinogens and remain risk drivers at many contaminated sites. The projected costs to remedy the estimated 113,000 sites in the United States alone exceed $200 billion (3). Bioremediation has emerged as a cost-efficient remedial technology, especially when implemented as a monitored natural attenuation (MNA) approach. Underlying this promising technology is the discovery of organohalide-respiring bacteria, which use chlorinated compounds as terminal electron acceptors to fuel their energy metabolism and form nontoxic products (4–6).

A variety of bacteria (e.g., *Dehalobacter*, *Desulfitobacterium*, *Sulfurospirillum*, and *Geobacter*) can utilize tetrachloroethene (PCE) and/or trichloroethene (TCE) as electron acceptors, leading to the formation of predominantly *cis*-1,2-dichloroethene (*c*DCE) (7–10). Preferential formation of *trans*-1,2-dichloroethene (*t*DCE) and 1,1-dichloroethene (1,1-DCE) has also been reported (11, 12). In contrast to the microbial diversity implicated in the formation of DCEs, the dechlorination of DCEs and vinyl chloride (VC) to environmentally benign ethene has been limited to strains of the species *Dehalococcoides mccartyi* with VC reductive dehalogenases (RDases) (e.g., BvcA, VcrA, and TceA) (13–17). *D. mccartyi* strains 195 and FL2 expressing TceA convert *c*DCE and 1,1-DCE to VC, but growth with VC as electron acceptor was uncertain (4, 18, 19). Recent work demonstrated that *D. mccartyi* strains expressing TceA grow with VC as electron acceptor as long as sufficient cobamide (e.g., vitamin B_{12}) is provided (17). *D. mccartyi* strain VS expressing VcrA utilizes *c*DCE, 1,1-DCE, and VC as electron acceptors but not *t*DCE (13, 20, 21). The BvcA RDase of *D. mccartyi* strain BAV1 was demonstrated to convert all three DCE isomers to VC and VC to ethene (14, 22). The recognition that *D. mccartyi* can promote ethene formation triggered the development of commercial bioaugmentation cultures (e.g., KB-1 and SDC-9) that rely on *D. mccartyi* strains carrying VC RDase genes to achieve detoxification (23). The quantitative monitoring of *D. mccartyi* and VC RDase gene abundances has become a cornerstone approach in environmental monitoring regimens to predict ethene formation at bioremediation sites (24, 25). Although *D. mccartyi* strains are keystone bacteria for detoxification of chlorinated ethenes, environmental monitoring data indicate ethene formation at sites where *D. mccartyi* and the known VC RDase genes are absent or present in low abundance (25), suggesting that other, not yet identified, microorganisms contribute to reductive dechlorination of VC.

The genus *Dehalogenimonas* is phylogenetically related to the genus *Dehalococcoides* within the class *Dehalococcoidia* (26). Similar to *D. mccartyi*, members of the genus *Dehalogenimonas* were characterized as obligate organohalide-respiring bacteria with the ability to utilize chlorinated alkanes as electron acceptors. *Dehalogenimonas lykanthroporepellens* strain BL-DC-9[T], *Dehalogenimonas alkenigignens* strain IP3-3[T], *Dehalogenimonas formicexedens* strain NSZ-14[T], and *Dehalogenimonas alkenigignens* strain BRE15M couple growth with the reductive dehalogenation of a variety of polychlorinated alkanes, including 1,2-dichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, 1,2-dichloropropane, and 1,2,3-trichloropropane (26–32). Similar to *D. mccartyi* strains, members of the *Dehalogenimonas* genus are heterotrophs and require acetate as the carbon source for growth (27, 28, 33). While *D. mccartyi* strains strictly require H_2, the characterized *Dehalogenimonas* species utilize H_2 and formate as electron donors (31).

In a 1,1,2,2-tetrachloroethane-to-ethene dechlorinating mixed culture derived from contaminated wetland sediment, *Dehalogenimonas* sp. strain WBC-2 was identified as the population responsible for reductive dechlorination of *t*DCE to VC. This *Dehalogenimonas* strain did not dechlorinate VC, and VC-to-ethene reductive dechlorination in this culture
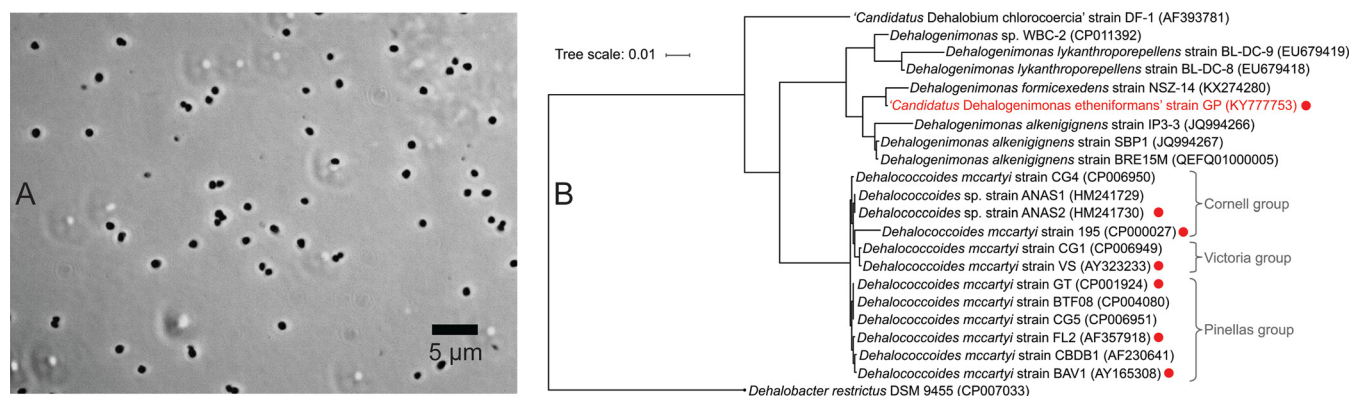
FIG 1 (A) Phase-contrast microscopic image of strain GP grown with VC as the electron acceptor, formate as the electron donor, and acetate as the carbon source. (B) Phylogenetic tree of strain GP based on the nearly full-length 16S rRNA genes (NCBI gene accession numbers are shown in parentheses). The scale bar represents 0.01 substitutions per nucleotide site. Isolates able to reductively dechlorinate VC to ethene are marked with solid red circles.

was mediated by a *D. mccartyi* strain carrying the *vcrA* RDase gene (34, 35). A TCE-to-ethene dechlorinating enrichment culture was derived from grape pomace collected from a pristine environment without prior exposure to chlorinated solvents. Intriguingly, the enrichment culture did not contain *D. mccartyi*, and "*Candidatus* Dehalogenimonas etheniformans" strain GP was implicated in the observed reductive dechlorination reactions (36). We isolated strain GP from the TCE-to-ethene dechlorinating enrichment culture to characterize key physiological features of this novel bacterium capable of reductive dechlorination of TCE, all DCE isomers, and VC to environmentally benign ethene.

RESULTS

Cell morphology and phylogeny of strain GP. Repeated dilution-to-extinction series and transfers in medium with VC as electron acceptor and amended with the peptidoglycan biosynthesis inhibitors ampicillin and vancomycin resulted in a microscopically homogeneous culture (Fig. 1A). No growth was observed without VC or in medium amended with yeast extract. Whole-genome sequencing (WGS) did not provide evidence for the presence of contaminating sequences (37). "*Candidatus* Dehalogenimonas etheniformans" strain GP forms round, coccus-shaped cells with an average cell size of $0.71 \pm 0.09 \ \mu$m ($n = 30$) (Fig. 1A). Phylogenetic analysis based on the nearly full-length 16S rRNA gene sequences revealed that strain GP is affiliated with the genus *Dehalogenimonas* within the class *Dehalococcoidia* and is closely related to *Dehalogenimonas formicexedens* strain NSZ-14^T (Fig. 1B).

Organohalide respiration with DCEs and VC. With H_2 provided as the electron donor, axenic strain GP cultures converted $66.9 \pm 4.6 \ \mu$mol of VC to a stoichiometric amount of ethene ($67.5 \pm 5.6 \ \mu$mol) within 14 days, and a maximum dechlorination rate of $182.8 \pm 3.0 \ \mu$mol L^{-1} day^{-1} was observed (Fig. 2A). When formate was supplied as the sole electron donor, VC ($81.3 \pm 10.1 \ \mu$mol) was completely dechlorinated to ethene ($75.0 \pm 10.4 \ \mu$mol) within 17 days, and a maximum dechlorination rate of $174.6 \pm 10.2 \ \mu$mol L^{-1} day^{-1} was determined (Fig. 2C). During reductive dechlorination of VC to ethene, $84.0 \pm 6.0 \ \mu$mol of formate was consumed, which approximately equaled the amount of VC transformed to ethene (Fig. 2C). The measured stoichiometry (without considering the reducing equivalents consumed for cell synthesis) demonstrated that the electrons generated from formate oxidation were used for reductive dechlorination of VC to ethene according to equation 1,

$$HCOO^- + H_2C = CHCl \rightarrow H_2C = CH_2 + CO_2 + Cl^- \qquad (1)$$

In medium bottles inoculated with strain GP but lacking an electron donor (i.e., H_2 or formate), VC was not dechlorinated and no ethene was formed (data not shown).

In strain GP cultures grown with H_2 and VC, the copy numbers of the 16S rRNA gene and the VC RDase gene *cerA* increased from $(6.8 \pm 2.8) \times 10^6$ to $(1.7 \pm 0.3) \times 10^8$
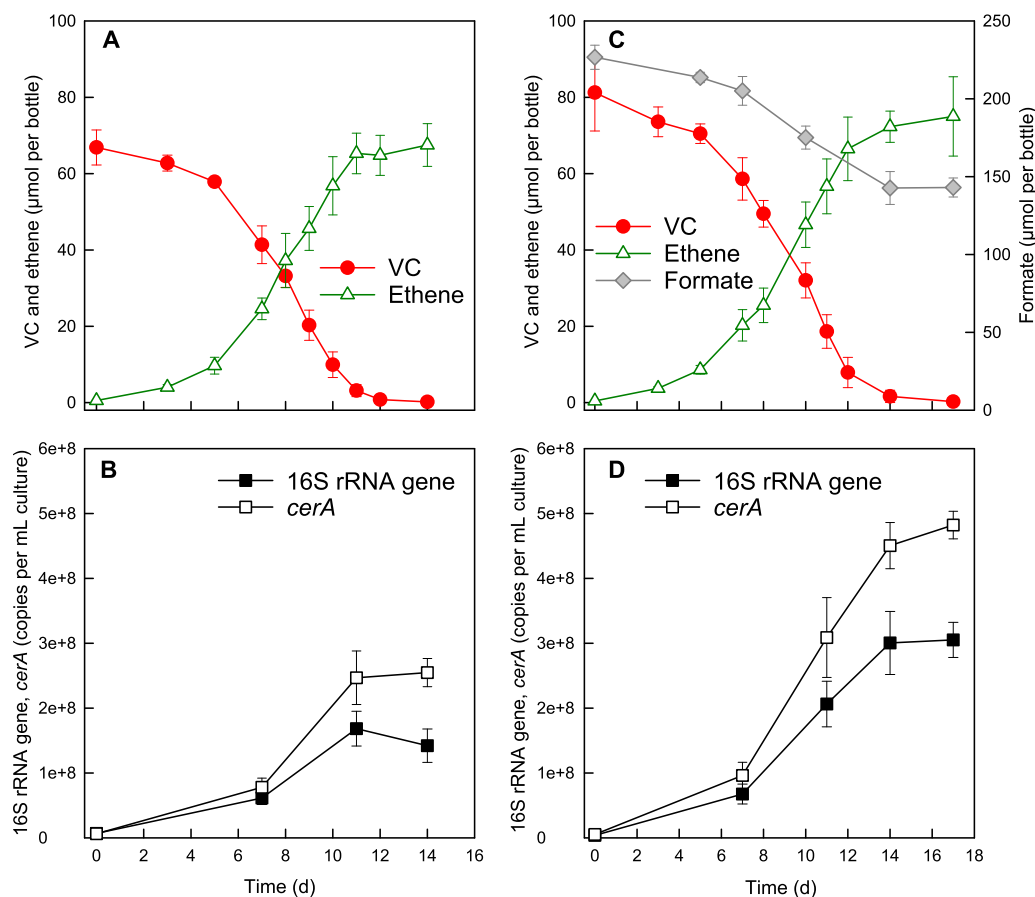
FIG 2 Reductive dechlorination of VC to ethene in strain GP cultures with H_2 (A) or formate (C) as the electron donor and the corresponding increase of strain GP 16S rRNA gene and *cerA* gene copies (B and D) during the growth period. Acetate was provided as the carbon source. The data represent the average values of triplicate incubations, and the error bars represent the standard deviations. Error bars are not shown when smaller than the symbol size.

and from $(6.3 \pm 2.2) \times 10^6$ to $(2.6 \pm 0.2) \times 10^8$, respectively (Fig. 2B). Growth yields of $(1.2 \pm 0.2) \times 10^8$ and $(1.9 \pm 0.6) \times 10^8$ cells per μmol VC consumed were determined based on 16S rRNA gene and *cerA* gene enumeration, respectively, both of which exist as single-copy genes in the genome of strain GP. In formate/VC-grown cultures, the copy numbers of the strain GP 16S rRNA gene and the *cerA* gene increased from $(3.8 \pm 1.9) \times 10^6$ to $(3.1 \pm 0.3) \times 10^8$ and from $(5.2 \pm 3.9) \times 10^6$ to $(4.8 \pm 0.2) \times 10^8$, respectively (Fig. 2D). Growth yields of $(1.9 \pm 0.2) \times 10^8$ and $(2.9 \pm 0.1) \times 10^8$ cells per μmol VC consumed were measured based on the enumeration of the 16S rRNA gene and the *cerA* gene, respectively. Based on the 16S rRNA gene qPCR data (Fig. 2B and D), doubling times of 2.75 and 3.25 days with H_2 and formate, respectively, as the electron donor were determined for strain GP during growth with VC as the electron acceptor.

When both formate and H_2 were amended simultaneously, strain GP cultures converted 78.2 ± 6.1 μmol of VC to 79.5 ± 5.8 μmol of ethene and consumed 37.9 ± 2.3 μmol of H_2 and 46.8 ± 3.3 μmol of formate during a 15-day incubation period (Fig. 3). The two electron donors were concurrently consumed at similar rates as long as VC was provided as the electron acceptor. VC was degraded at a maximum rate of 254.1 ± 39.5 μmol L^{-1} day^{-1}, which was greater than in cultures that received only H_2 or only formate as the electron donor (Fig. 3).

In the first generation transfer cultures without acetate addition, VC dechlorination and growth of strain GP were observed (see Fig. S1 in the supplemental material). High-performance liquid chromatography (HPLC) measurements revealed that approximately 0.2
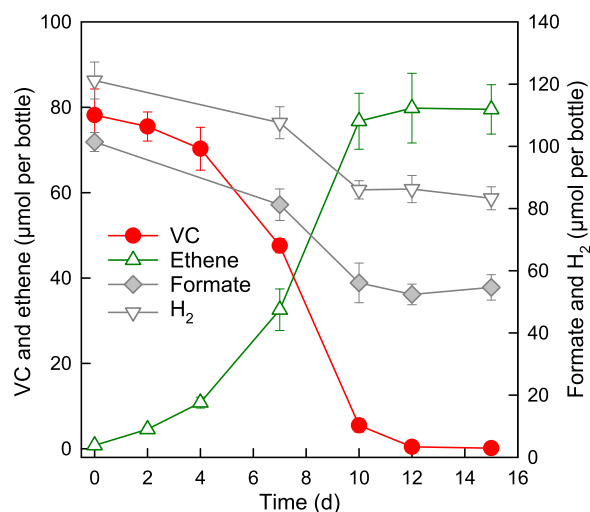
FIG 3 Concurrent consumption of H$_2$ and formate by strain GP with VC provided as the electron acceptor. Acetate was provided as the carbon source. The data represent the average values of triplicate incubations, and the error bars represent the standard deviations. Error bars are not shown when smaller than the symbol size.

mM acetate (~10 μmol total per bottle) transferred with the inoculum was completely consumed during the growth period (see Fig. S1). In the second generation transfer cultures without acetate amendment, no VC dechlorination or growth of strain GP was observed; however, both ethene formation and growth resumed following the addition of 1 mM acetate after a 12-day incubation period without growth or dechlorination activity (see Fig. S2). Apparently, "*Candidatus* Dehalogenimonas etheniformans" strain GP is a heterotrophic bacterium and acetate is required as a carbon source to support growth with VC as the electron acceptor and H$_2$ or formate as the electron donor.

In addition to VC, "*Candidatus* Dehalogenimonas etheniformans" strain GP utilized TCE and all three DCE isomers (i.e., 1,1-DCE, *c*DCE, and *t*DCE) as terminal electron acceptors with formate or H$_2$ as the electron donor and acetate as the carbon source (Fig. 4). VC was transiently formed when a DCE isomer was provided as the electron acceptor, and the final product was ethene (Fig. 4A to C). Interestingly, with TCE as the electron acceptor, all three DCE isomers were formed concomitantly prior to the conversion to VC and ethene (Fig. 4D). Maximum amounts of 6.3 $\pm$ 2.1 and 1.9 $\pm$ 0.2 μmol of *c*DCE and 1,1-DCE, respectively, were measured, and *t*DCE amounts never exceeded 0.2 $\pm$ 0.1 μmol per bottle (Fig. 4D). PCE was not dechlorinated and inhibited reductive dechlorination of other chlorinated ethenes in strain GP cultures. A PCE concentration of ~0.08 mM PCE (~9.8 μmol per bottle) prevented VC reductive dechlorination activity (data not shown).

Carbon monoxide formation in VC-grown strain GP cultures. During dechlorination and growth with VC as the electron acceptor and formate as the electron donor, a continuous buildup of carbon monoxide (CO) was observed in strain GP cultures (Fig. 5). After two amendments of 94.9 $\pm$ 10.2 and 87.7 $\pm$ 4.0 μmol of VC, a total amount of 0.23 $\pm$ 0.01 μmol CO (equivalent to 51.4 $\pm$ 1.2 ppmv CO in the headspace of the cultivation bottles) was measured after a 21-day incubation period (Fig. 5). CO formation was also observed in cultures in which H$_2$ replaced formate as electron donor. Externally amended CO (4 μmol, equivalent to ~880 ppmv in the cultivation vessel) strongly inhibited VC degradation by strain GP (see Fig. S3).

Effects of salinity and temperature on reductive dechlorination of VC. Elevated salinity impeded reductive dechlorination of VC to ethene in strain GP cultures (Fig. 6). In standard basal salt medium containing 1 g L^{-1} NaCl, strain GP completely converted VC (83.8 $\pm$ 3.1 μmol) to ethene (86.0 $\pm$ 2.4 μmol) over a 10-day incubation period, at a rate of 246.0 $\pm$ 8.5 μmol L^{-1} day^{-1}. When the NaCl concentration in the medium was increased to 2.5 and 5 g L^{-1}, the dechlorination rates decreased to 189.2 $\pm$ 4.6 and 128.5 $\pm$
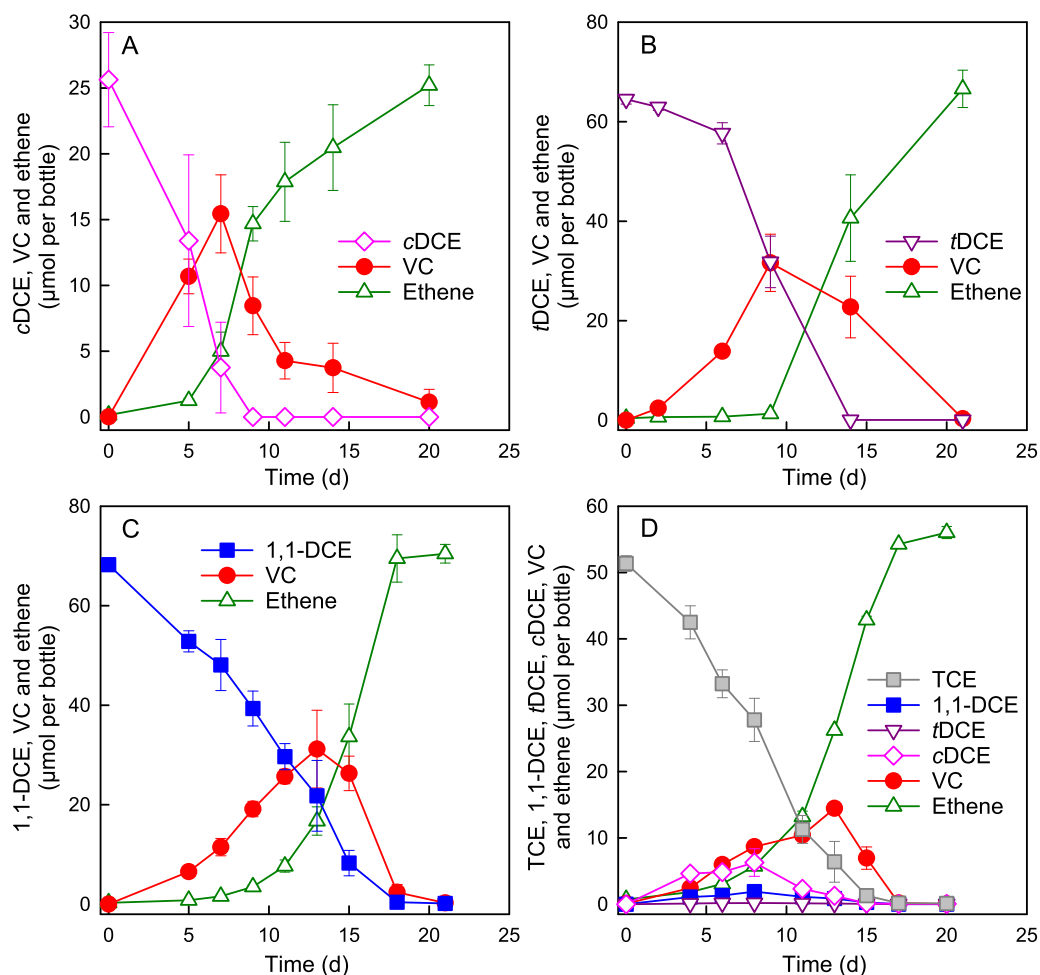
FIG 4 Reductive dechlorination of *c*DCE (A), *t*DCE (B), 1,1-DCE (C), and TCE (D) in strain GP cultures with formate as the electron donor and acetate as the carbon source. The data represent the average values from triplicate incubations, and the error bars represent the standard deviations. Error bars are not shown when smaller than the symbol size.

11.4 μmol L^{-1} day^{-1}, respectively, and the time required to achieve complete dechlorination of VC to ethene increased to 14 and 21 days, respectively (Fig. 6). A further decline in dechlorination performance was observed at NaCl concentrations of 7.5 and 10 g L^{-1}, and the degradation rates decreased to 20.9 $\pm$ 0.2 and 4.8 $\pm$ 0.4 μmol L^{-1} day^{-1}, respectively. Under these higher NaCl conditions, the dechlorination process stalled, leading to incomplete VC-to-ethene reductive dechlorination even after an extended incubation period of 81 days (Fig. 6). No VC-to-ethene dechlorination activity was observed at an NaCl concentration of 15 g L^{-1} (Fig. 6), and no growth occurred.

The highest rates of VC dechlorination were observed in cultures incubated at 30°C and 25°C (Fig. 7). Longer incubation periods were required to achieve complete conversion of VC to ethene at 20°C and 15°C (Fig. 7). No dechlorination activity was observed during a 58-day incubation period when strain GP cultures were incubated at 4°C.

DISCUSSION

The presence of a strictly organohalide-respiring bacterium in an environment (i.e., grape pomace) without prior exposure to anthropogenic chlorinated compounds, including chlorinated solvents, may seem perplexing; however, there is mounting evidence that natural processes generate chloroorganics, including chlorinated ethenes (38). For example, VC was detected in soil gas samples at concentrations significantly exceeding those measured
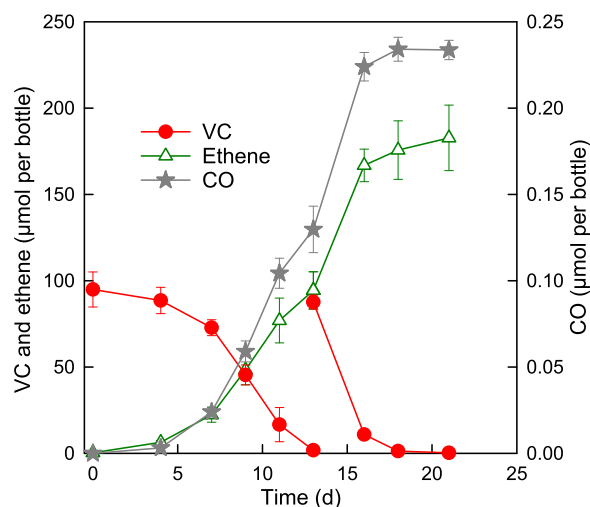
FIG 5 Formation and accumulation of CO in strain GP cultures during reductive dechlorination of VC to ethene with formate as the electron donor and acetate as the carbon source. A second amendment of VC occurred after VC depletion at 13 days. The data represent the average values from triplicate incubations, and the error bars represent the standard deviations. Error bars are not shown when smaller than the symbol size.

in ambient air, suggesting VC formation in soil (39). It has been documented that reactions between soil organic matter, chloride ions, and oxidants (e.g., ferric ions and hydroxyl radicals) lead to the formation of volatile chlorinated halocarbons such as VC (39). Organohalide respiration with VC as the electron acceptor has so far been ascribed exclusively to certain *Dehalococcoides mccartyi* strains, including the *D. mccartyi* isolates BAV1, VS, GT, 195, and FL2 (15, 17). The presence and activity of *D. mccartyi* strains have been linked to ethene formation, and *in situ* monitoring of *D. mccartyi* 16S rRNA genes and the VC RDase genes (e.g., *tceA*, *vcrA*, and *bvcA*) provide valuable information for management of contaminated sites. The current study demonstrates that a non-*D. mccartyi* bacterial isolate can perform respiratory reductive dechlorination of TCE, DCEs and VC, and the new findings expand our understanding of the physiology of the genus *Dehalogenimonas*.

The characterized *D. mccartyi* strains utilize only H_2 and not formate as the electron donor for reductive dechlorination. *Dehalogenimonas formicexedens* strain NSZ-14[T], *Dehalogenimonas lykanthroporepellens* strain BL-DC-9[T], and *Dehalogenimonas alkenigignens* strain IP3-3[T] utilize formate, in addition to H_2, as the electron donor for reductive

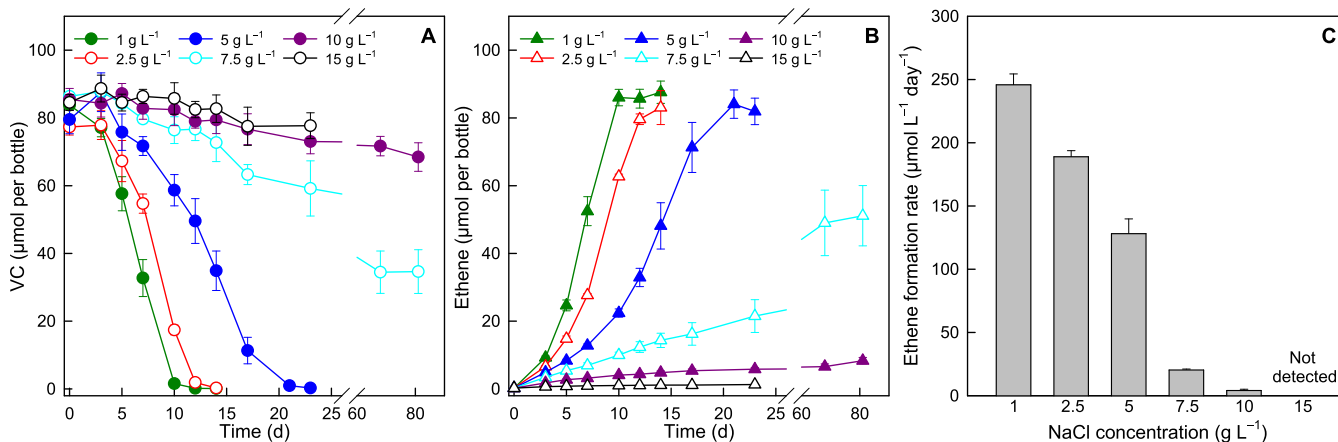**FIG 6** Effect of salinity on reductive dechlorination of VC to ethene in strain GP cultures. VC degradation (A), ethene formation (B), and rates of ethene formation (C) in strain GP cultures under different salinity conditions are shown. The data represent the average values of triplicate incubations, and the error bars represent the standard deviations. Error bars are not shown when smaller than the symbol size.

FIG 7 Effect of temperature on reductive dechlorination of VC to ethene in strain GP cultures. Day 0 measurements were performed after the cultures had been incubated at the respective temperatures for 5 h. Complete equilibrium of VC between the gas and aqueous phases was not achieved in cultures incubated at 4°C, which is a likely explanation for the apparent increase in VC. The data represent the average values of triplicate incubations, and the error bars represent the standard deviations. Error bars are not shown when smaller than the symbol size.
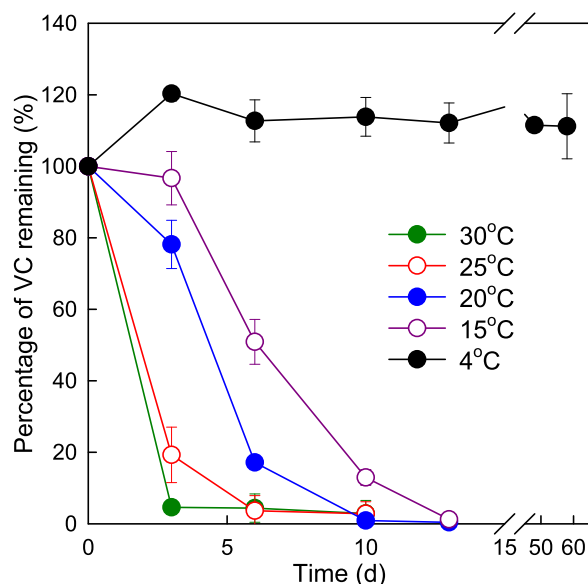
dechlorination (31). Similar to other *Dehalogenimonas* strains, "*Candidatus* Dehalogenimonas etheniformans" strain GP utilizes both H_2 and formate as electron donors. An interesting observation was made in VC-grown strain GP cultures when formate replaced H_2 as the electron donor. The amount of H_2 increased from 0.24 ± 0.02 to 0.54 ± 0.02 μmol (equal to 53.9 ± 5.1 to 119.1 ± 4.2 ppmv in the headspace of the cultivation vessels) (see Fig. S4 in the supplemental material), suggesting that strain GP might convert formate to H_2 and H_2 serves as the direct electron donor for reductive dechlorination. H_2 formation did not occur in strain GP cultures without VC or in medium with VC but without inoculum (see Fig. S4). Similar VC degradation rates were observed in strain GP cultures using H_2 or formate as the electron donor (Fig. 2). When both formate and H_2 were present in strain GP cultures, similar amounts of formate and H_2 were consumed (i.e., 37.9 ± 2.3 and 46.8 ± 3.3 μmol, respectively) (Fig. 3). Apparently, strain GP concomitantly consumes formate and H_2 at similar rates, suggesting that the organism does not prefer one electron donor over the other. Two formate dehydrogenases (GenBank locus tags QNT75850 and QNT76458) are encoded in the strain GP genome (GenBank accession number CP058566.2), consistent with the organism's capacity for formate oxidation (37).

Coccoid cells about 0.7 μm in diameter were observed with phase-contrast microscopy (Fig. 1A), consistent with recent cryo-electron tomography imaging of strain GP (40). The cell size and shape of strain GP are consistent with other reports characterizing *Dehalogenimonas* isolates (28, 41), indicating that the coccoid cell shape of *Dehalogenimonas* cells is a distinguishing characteristic, compared with the disc-shaped morphology of *D. mccartyi* cells (15). A doubling time of 2.75 days was observed for strain GP with VC as electron acceptor and H_2 as electron donor. *Dehalogenimonas lykanthroporepellens* strains BL-DC-9[T] and BL-DC-8 have reported doubling times of 4.1 and 4.8 days, respectively, during growth with 1,2,3-trichloropropane and H_2 (27). Shorter doubling times, ranging from 0.8 to 3 days, have been reported for *D. mccartyi* strains (15), and VC-grown *D. mccartyi* strains BAV1 and GT have doubling times of 2.2 and 2 to 2.5 days, respectively (5, 42). A doubling time of 1.7 days was reported for *D. mccartyi* strain VS during growth with VC in a mixed culture (20).

The growth yield of strain GP cultures grown with VC as electron acceptor was slightly higher with formate than with H_2 as electron donor, and $(1.9 \pm 0.2) \times 10^8$ versus

$(1.2 \pm 0.2) \times 10^8$ (based on 16S rRNA gene enumeration) and $(2.9 \pm 0.1) \times 10^8$ versus $(1.9 \pm 0.6) \times 10^8$ (based on *cerA* gene enumeration) cells per μmol of VC consumed, respectively, were generated (Fig. 2B and D). A possible explanation for the slightly higher growth yield with formate versus H$_2$ is the ability of strain GP to conserve energy released from formate oxidation, which is about -3.4 kJ per mol of formate under standard conditions. The growth yields measured for "*Ca.* Dehalogenimonas etheniformans" strain GP were comparable to those observed for *D. mccartyi* strains, which have been reported to range between 6.3×10^7 and 5.2×10^8 cells per μmol of VC dechlorinated (5, 17, 20, 42).

The growth yields determined with qPCR targeting the *cerA* gene were consistently $\sim$1.5-fold higher than those measured with the 16S rRNA gene-targeted assay (Fig. 2B and D), which was surprising because the analysis of the strain GP genome indicated that both the 16S rRNA gene and the *cerA* gene are single-copy genes (37). Calibration curves generated with the synthetic linear DNA fragment containing the target regions of both the 16S rRNA gene and the *cerA* gene were highly similar (see Fig. S5), demonstrating that the two qPCR assays amplify their respective target gene with similar efficiencies (i.e., 93%) (see Fig. S5). Interestingly, *cerA* gene reads outnumbered 16S rRNA gene reads in genome-derived Illumina WGS reads and followed a similar trend as the qPCR results. Aligning WGS reads to genes derived from the GP genome (GenBank accession number CP058566) revealed that the depth of coverage of *cerA* is 1.33-fold higher than that of the 16S rRNA gene ($56.5\times$ versus $42.5\times$) and 1.44-fold higher than the average gene coverage ($39.2\times$). Figure S6 in the supplemental material shows the average gene read depth of genes across the strain GP genome, illustrating that *cerA* belongs to a group of colocalized genes with above-average read depth, reaching up to $\sim$80$\times$, approximately double that of the average gene. Interestingly, qPCR measurements at field sites demonstrated that *D. mccartyi* RDase gene copies can exceed the *D. mccartyi* cell numbers, based on 16S rRNA gene measurements, 10- to 10,000-fold (25), and it was speculated that RDase genes can occur on gene transfer agents (25, 43). A recent cryo-electron tomography imaging study revealed extracellular lipid vesicles in axenic strain GP cultures (40). While the presence of DNA in such vesicles has yet to be demonstrated, it is conceivable that these vesicles are enriched in *cerA* but lack the 16S rRNA gene. These observations may explain the incongruous qPCR data; however, the biological meaning of the observed variations in gene abundances in *Dehalococcoidia* cultures and the potential role of the observed extracellular lipid vesicles remain to be determined.

In cultures of *D. mccartyi* strain 195, CO accumulation to a maximum amount of approximately 5 μmol was reported after repeated additions of TCE ($\sim$385 μmol total) (44). Consistent with this observation, CO (approximately 0.23 μmol per bottle) formed and accumulated in strain GP cultures following the consumption of $\sim$180 μmol of VC (Fig. 5). Both *D. mccartyi* strain 195 and "*Ca.* Dehalogenimonas etheniformans" strain GP have an incomplete Wood-Ljungdahl pathway (37, 44). Isotopic labeling experiments using [^{13}C]acetate revealed that the CO formed in *D. mccartyi* strain 195 cultures was derived from the carboxyl group of acetate (44). The CO dehydrogenase (CODH) subunit of the CODH/acetyl-coenzyme A (CoA) decarbonylase-synthase (ACDS) complex is missing in the genomes of both *D. mccartyi* strain 195 and "*Ca.* Dehalogenimonas etheniformans" strain GP. Therefore, the ACDS complex could catalyze the decarbonylation reaction of acetyl-CoA, generating CO, but oxidation to CO$_2$ is not possible due to the absence of a CODH subunit. The genomes of *D. mccartyi* strains 195, BAV1, FL2, GT, VS, and CBDB1 and *Dehalogenimonas* species strains BL-DC-9^T, IP3-3^T, NSZ-14, and WBC-2 all possess ACDS but lack CODH, suggesting that CO formation during metabolic reductive dechlorination might be a common phenomenon in these organohalide-respiring bacteria. CO inhibition of *D. mccartyi* growth has been reported (44), and CO also inhibits growth of strain GP (see Fig. S3). CO has a high binding affinity for metals and likely affects metalloenzymes, such as Fe-containing hydrogenases, a plausible explanation for the observed inhibition (45). The toxicity of CO can complicate axenic cultivation of *Dehalococcoidia*, and

cocultivation with a CO-consuming partner population demonstrated enhanced growth of *D. mccartyi* strain 195 (44, 46).

Bioremediation of saline groundwater near coastal areas with freshwater-derived bioaugmentation consortia can be challenging because the characterized *D. mccartyi* strains are sensitive to elevated salt concentrations (47, 48). Similar observations were made with strain GP, and elevated NaCl concentrations decreased the rate of reductive dechlorination of VC to ethene (Fig. 6). *Dehalogenimonas lykanthroporepellens* strain BL-DC-9^T survives moderate saline conditions, and growth occurred at NaCl concentrations of up to 20 g L^{-1} (2% [w/v], or 20 ppt), which was explained by multiple osmotic stress response mechanisms (28, 41). Strain GP grew and completely dechlorinated VC to ethene with 5 g L^{-1} NaCl; however, when salinity increased to 10 g L^{-1} NaCl, incomplete VC dechlorination was observed (Fig. 6). Apparently, strain GP performs reductive dechlorination in freshwater and slightly to moderately saline environments with salinity not exceeding 10 g L^{-1} NaCl. Freshwater-derived *D. mccartyi* and *Dehalogenimonas* strains have limited salinity tolerance, but the enrichment of organohalide-respiring *Dehalococcoidia* strains from polluted estuarine and marine sediments indicates that strains with elevated salt tolerance exist (49–51).

Groundwater temperatures generally correlate with climate zones, and the average temperature of groundwaters in the United States ranges between 3 and 25°C (52). Temperature affects the rates of microbial processes, including biodegradation. *Dehalogenimonas lykanthroporepellens* strains BL-DC-8 and BL-DC-9^T perform organohalide respiration over a temperature range from 20 to 34°C (optimum temperature, 28 to 34°C) (27, 28). *Dehalogenimonas alkenigignens* strains IP3-3^T and SBP-1 reportedly have a broader temperature range spanning 18 to 42°C (optimum temperature, 30 to 34°C) (30). Dechlorination activity of *Dehalogenimonas formicexedens* strain NSZ-14^T, the closest relative of strain GP, occurred over a temperature range of 20 to 37°C (optimum temperature, 30 to 34 °C) but not at temperatures of ≤15°C or ≥42°C (31). Strain GP completely dechlorinated VC to ethene at 15°C, but ethene formation was not apparent at 4°C (Fig. 7). Similar to *Dehalogenimonas* isolates, *D. mccartyi* strains are mesophiles with an optimum growth temperature between 25 and 30°C, and dechlorination occurred over a temperature range of 15 to 35°C (15). The *D. mccartyi*-containing PCE-to-ethene dechlorinating consortium KB-1 was reported to dechlorinate TCE to ethene between 10 and 30°C (53). *c*DCE and VC stalled in sand-packed columns bioaugmented with KB-1 when the incubation temperature was 15°C, whereas ethene formation was observed in replicate columns operated at 35°C (54). The temperature range of 10 to 15°C appears to be a boundary for *D. mccartyi* and *Dehalogenimonas* strains to complete dechlorination of DCEs and VC to ethene under laboratory conditions. A study employing ^{14}C-labeled chlorinated ethenes reported degradation in microcosms established with cold temperature-adapted aquifer and river sediments, suggesting that the microbial reductive dechlorination process can operate at lower temperatures (55).

pH is another parameter impacting the microbial reductive dechlorination process. *D. mccartyi* strains perform reductive dechlorination between pH 6.5 and pH 8.0 (15), but sustained (i.e., growth-linked) organohalide respiration activity is not expected at pH values below 5.5 (56, 57). *Dehalogenimonas lykanthroporepellens* strains BL-DC-9^T and BL-DC-8 and *Dehalogenimonas alkenigignens* strain IP3-3^T exhibited reductive dechlorination in the pH range of 6.0 to 8.0 but not at pH values of ≤5.5 or ≥8.5 (27, 28, 30). The dechlorination activity of *Dehalogenimonas formicexedens* strain NSZ-14^T, the closest relative of strain GP, occurred at pH values between 5.5 and 7.5 (31). Although we did not systematically investigate the response of strain GP to pH, preliminary data suggested that no growth occurred below pH 5.5.

Implications for bioremediation. Conversion of the human carcinogen VC to environmentally benign ethene is a key step for achieving detoxification at sites where the microbial reductive dechlorination process has been implemented as a site remedy for chlorinated ethenes. To date, this process has been attributed exclusively to certain *D. mccartyi* strains, which has impacted the development of bioaugmentation consortia

and bioremediation monitoring tools with consequences for site management decision-making, including that regarding site closures. The finding that at least some *Dehalogenimonas* species utilize TCE, DCEs, and VC as growth-supporting electron acceptors and rely on VC RDases not reported to occur in *D. mccartyi* impacts the interpretation of geochemical and molecular data from contaminated sites. Survey data revealed that *Dehalogenimonas* 16S rRNA genes are frequently detected at chlorinated solvent sites and often outnumber *D. mccartyi* (36), suggesting that *Dehalogenimonas* species are major contributors to the reductive dechlorination process. The recognition that *Dehalogenimonas* contribute to the detoxification of chlorinated ethenes may lead to the development of more effective bioaugmentation consortia that rely on both *D. mccartyi* and *Dehalogenimonas* populations. *D. mccartyi* strictly require H_2 as electron donor, but delivering H_2 to subsurface environments is challenging. A common practice is the addition of fermentable substrates (e.g., emulsified vegetable oil or molasses) to increase the flux of H_2; however, secondary impacts such as excessive methanogenesis and groundwater acidification can impact groundwater quality (56, 58). In addition to H_2, *Dehalogenimonas* populations utilize water-soluble formate as an electron donor, a physiological feature that may offer opportunities for more targeted and efficient approaches to deliver and distribute electron donor in the contaminated subsurface. The description of "*Candidatus* Dehalogenimonas etheniformans" expands the bacterial diversity capable of detoxification of chlorinated ethenes, and future studies should explore whether synergisms between organohalide-respiring *D. mccartyi* and *Dehalogenimonas* strains can achieve more efficient bioremediation treatment.

MATERIALS AND METHODS

Chemicals. VC (purity, >99%) was purchased from SynQuest Laboratories (Alachua, FL, USA). PCE (>99%) was purchased from Acros (Morris Plains, NJ, USA). TCE (>98.0%), 1,1-DCE (>99.5%), *c*DCE (>96.0%), *t*DCE (>99.9%), and ethene (>99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). CO gas (>99.0%) was purchased from Sigma-Aldrich. All other chemicals used in this study were reagent grade or of higher purity.

Enrichment, isolation, microscopy, and phylogenetic analysis. An enrichment culture capable of dechlorinating VC, DCEs, and TCE to ethene was established with grape pomace compost as the source material (36). Enrichment culture GP was transferred every 4 to 6 weeks for over 3 years in defined mineral salt medium containing 1 g L^{-1} ampicillin and 0.1 g L^{-1} vancomycin, acetate as the carbon source, H_2 as the electron donor, and VC as the electron acceptor. Repeated dilution-to-extinction series in liquid mineral salt medium were performed as described (59–61) and achieved isolation of strain GP.

The presence of contaminating microorganisms was assessed by transfers to nutrient-rich medium (i.e., with the amendment of 0.1% [w/v] yeast extract) and regularly examination of cultures with a Zeiss AX10 light microscope (Jena, Germany). WGS combined both NovaSeq and Nanopore sequencing technologies (37) and provided no evidence for the presence of contaminating sequences. The size of strain GP cells (n = 30) was estimated based on analysis of the cell micrographs (1,000-fold magnification) using AxioVision SE64 (release 4.9.1) software.

For phylogenetic analysis, 16S rRNA gene sequences of representative dechlorinators were obtained from the SILVA database (62). The 16S rRNA gene sequence of strain GP was retrieved from the genome assembly (37). The sequences were aligned using the SINA aligner (63). The phylogenetic tree was computed using the RAxML program (v8) (64) through SILVA Alignment, Classification, and Tree Service (ACT) and visualized using iTOL (v5) (65).

Cultivation of "*Candidatus* Dehalogenimonas etheniformans" strain GP. Routine cultivation of strain GP occurred in 160-mL glass serum bottles containing 50 mL of bicarbonate-buffered (30 mM) basal mineral salt medium (pH 7.3) reduced with 0.2 mM Na_2S, 0.2 mM L-cysteine, and 0.5 mM sodium dithiothreitol (59–61). The bottles were sealed with butyl rubber stoppers (Bellco Glass Inc., Vineland, NJ, USA) under a headspace of N_2/CO_2 (80:20 [vol/vol]) and provided with 5 mM formate or 6 mL H_2 (~246 μmol) as the electron donor and 2 mL VC (~82 μmol) as the electron acceptor. Acetate (1 mM) served as the carbon source. All cultures received 5% (v/v) inocula and were incubated at 30°C in the dark without agitation. Other chlorinated ethenes, including PCE (~50 μmol), TCE (~60 μmol), 1,1-DCE (~60 μmol), *c*DCE (~30 μmol), and *t*DCE (~60 μmol), were tested as the electron acceptors to support growth of strain GP with formate as the electron donor and acetate as the carbon source. To test whether acetate was required as carbon source, cultures were consecutively transferred without acetate. Growth of strain GP cultures was monitored by measuring the consumption of the chlorinated electron acceptors and the formation of dechlorination products (e.g., ethene). Cell numbers in strain GP cultures were determined by qPCR assays specifically targeting the 16S rRNA gene and the VC RDase gene *cerA* of strain GP (36), as described below. VC dechlorination and ethene formation rates were calculated based on the slopes of the linear regression lines of the VC consumption or ethene formation curves during the active growth phase (i.e., without considering the initial lag period and the transition to stationary phase) and are reported in μmol per liter per day. The doubling time t_d was calculated based on

TABLE 1 Primers and probes used for qPCR assays targeting the 16S rRNA gene and the *cerA* gene of strain GP

Primer/probe name	Target	Primer/probe sequence (5′ to 3′)[a]
Dhgm16S_478F	16S rRNA gene	AGCAGCCGCGGTAATACG
Dhgm16S_536R	16S rRNA gene	CCACTTTACGCCCAATAAATCC
Dhgm16S_500P	16S rRNA gene	FAM-AGGCGAGCGTTATC-MGB
cerA_1311F	*cerA* gene	GAGAATGCTTACAGACCTGCCT
cerA_1456R	*cerA* gene	AGTAAGGCGAGTCATAGCGTGG
cerA_1382P	*cerA* gene	FAM-CCTGTGGGATCTGTGCCGA-MGB

[a]FAM, 6-carboxyfluorescein; MGB, minor groove binder.

the equation $N = N_0 \times 2^{(t/t_d)}$, where N_0 and N are the initial and final cell numbers during the exponential growth phase, respectively, and t is the duration of the growth period.

Effects of salinity and temperature on VC reductive dechlorination. To test the effect of salinity on VC reductive dechlorination by strain GP, increasing amounts of NaCl were added to the medium to achieve concentrations of 2.5, 5, 7.5, 10, 15, 20, and 30 g L^{-1}. To test the effect of temperature on VC-to-ethene reductive dechlorination, strain GP cultures were grown with 5 mM formate (i.e., 250 μmol per bottle) and 2 mL VC (~82 μmol per bottle) at 30°C. Following complete VC consumption, triplicate strain GP cultures received 2 mL of VC, and were immediately incubated at temperatures of 30, 25, 20, 15, and 4°C. Aqueous samples (1 mL) were periodically withdrawn from the cultures at the respective incubation temperatures, immediately transferred to 20-mL glass autosampler vials, and analyzed by gas chromatography (GC) (see below). Aqueous samples were collected 5 h after VC addition initiated the experiment and served as the initial (i.e., time zero) measurements.

Real-time qPCR. Specific qPCR assays targeting the 16S rRNA gene and the VC RDase gene (*cerA*) were used to enumerate cell numbers and monitor the growth of strain GP. For DNA extraction, up to 5 culture suspension samples (1 mL) were collected over a growth cycle and filtered onto 0.22-μm Durapore membranes (Millipore, Cork, Ireland). DNA was extracted using the DNeasy PowerLyzer PowerSoil DNA isolation kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Primers and probes specifically targeting the 16S rRNA gene and *cerA* gene of strain GP were designed using Geneious Prime software (v11.0.6) (Table 1) and were optimized and validated following established procedures (66–68). qPCR analysis followed published protocols (66, 69) and was conducted using an Applied Biosystems QuantStudio 12K Flex real-time PCR system. Briefly, every 20-μL reaction mixture contained 10 μL of 2× Taqman Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA), 2 μL of DNA template (DNA concentrations between 20 and 100 ng μL^{-1}), and forward and reverse primers and probe at final concentrations of 300 nM each. The PCR thermal cycling protocol was as follows: 50°C for 2 min and then 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing and extension at 60°C for 1 min. Calibration curves used serial 10-fold dilutions of a LDFA1 synthetic linear DNA fragment (1,500 bp) with partial gene fragments of the strain GP *cerA* gene and the 16S rRNA gene, comprising the primer binding sites, and spanned a concentration range from 608 to 6.08 ×10^9 target gene copies per reaction. The LDFA1 linear DNA fragment was synthesized by Invitrogen (GeneArt Strings DNA fragments; Invitrogen, CA, USA).

Analytical procedures. Chlorinated ethenes and ethene were routinely quantified by manually injecting 0.1-mL headspace samples with 1-mL disposable plastic syringes with 25-gauge 7/8-inch needles (0.5 mm by 22 mm; Becton, Dickinson and Company, Franklin Lake, NJ, USA) into a 7890A GC system (Agilent, Santa Clara, CA, USA) equipped with a DB-624 column (length, 60 m; i.d., 0.32 mm; film thickness, 1.8 μm) and a flame ionization detector (FID), following established procedures (70). The inlet was maintained at 200°C, the oven temperature was kept at 60°C for 2 min followed by an increase to 200°C at a ramping rate of 25°C min^{-1}, and the FID was operated at 300°C. Samples collected during the temperature experiment were analyzed in 1-mL liquid samples using a GC-FID system equipped with an Agilent G1888 Network headspace autosampler. The headspace autosampler oven was set at 70°C, and the sample vials were equilibrated for 15 min prior to injection. The GC method was the same as described above for direct injection of headspace samples. Formate and acetate were analyzed on an Agilent 1200 series HPLC system equipped with an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operated at 30°C and a multiple-wavelength detector set to 210 nm. Operation was isocratic using 4 mM H$_2$SO$_4$ as eluent, at a flow rate of 0.6 mL min^{-1}. Aqueous samples (200 μL) were acidified with 4 μL of 1 M H$_2$SO$_4$ and filtered prior to HPLC analysis. The identification of peaks was based on retention times of authentic standards, and quantification was achieved using external calibration curves. To follow the consumption of H$_2$ in strain GP cultures, 1 mL of headspace sample was injected into an Agilent 3000A Micro-GC system (60). To measure CO and H$_2$ concentrations in strain GP cultures, 0.5 mL of headspace gas was injected into a Peak Performer 1 with a reducing compound photometer (Peak Laboratories, Mountain View, CA, USA) (71). The aqueous phase concentrations of CO and H$_2$ were calculated using Henry's law constants of 9.8 × 10^{-4} M atm^{-1} and 7.9 × 10^{-4} M atm^{-1}, respectively (72). A CO partial pressure of 1 ppmv in the headspace of strain GP cultures corresponded to a total amount of approximately 4.55 × 10^{-3} μmol of CO per bottle at 25°C, and 1 ppmv H$_2$ in the headspace corresponded to a total amount of 4.54 × 10^{-3} μmol of H$_2$ per bottle.

REFERENCES

1. Moran MJ, Zogorski JS, Squillace PJ. 2007. Chlorinated solvents in groundwater of the United States. Environ Sci Technol 41:74–81. https://doi.org/10.1021/es061553y.

2. McCarty PL. 2010. Groundwater contamination by chlorinated solvents: history, remediation technologies and strategies, p 1–28. *In* Stroo HF, Ward CH (ed), *In* situ remediation of chlorinated solvent plumes. Springer, New York, NY.

3. National Research Council. 2013. Alternatives for managing the nation's complex contaminated groundwater sites. National Academies Press, Washington, DC.

4. Maymó-Gatell X, Chien Y-T, Gossett JM, Zinder SH. 1997. Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. Science 276:1568–1571. https://doi.org/10.1126/science.276.5318.1568.

5. He J, Ritalahti KM, Yang K-L, Koenigsberg SS, Löffler FE. 2003. Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium. Nature 424:62–65. https://doi.org/10.1038/nature01717.

6. Löffler FE, Ritalahti KM, Zinder SH. 2013. *Dehalococcoides* and reductive dechlorination of chlorinated solvents, p 39–88. *In* Stroo HF, Leeson A, Ward CH (ed), Bioaugmentation for groundwater remediation. Springer, New York, NY.

7. Holliger C, Regeard C, Diekert G. 2003. Dehalogenation by anaerobic bacteria, p 115–157. *In* Häggblom MM, Bossert ID (ed), Dehalogenation: microbial processes and environmental applications. Springer US, Boston, MA.

8. Löffler FE, Cole JR, Ritalahti KM, Tiedje JM. 2003. Diversity of dechlorinating bacteria, p 53–87. *In* Häggblom MM, Bossert ID (ed), Dehalogenation: microbial processes and environmental applications. Springer US, Boston, MA.

9. Smidt H, de Vos WM. 2004. Anaerobic microbial dehalogenation. Annu Rev Microbiol 58:43–73. https://doi.org/10.1146/annurev.micro.58.030603.123600.

10. Atashgahi S, Lu Y, Smidt H. 2016. Overview of known organohalide-respiring bacteria—phylogenetic diversity and environmental distribution, p 63–105. *In* Adrian L, Löffler FE (ed), Organohalide-respiring bacteria. Springer, Berlin, Germany.

11. Griffin BM, Tiedje JM, Löffler FE. 2004. Anaerobic microbial reductive dechlorination of tetrachloroethene to predominately *trans*-1,2-dichloroethene. Environ Sci Technol 38:4300–4303. https://doi.org/10.1021/es035439g.

12. Zhang J, Joslyn AP, Chiu PC. 2006. 1,1-Dichloroethene as a predominant intermediate of microbial trichloroethene reduction. Environ Sci Technol 40:1830–1836. https://doi.org/10.1021/es051829m.

13. Müller JA, Rosner BM, von Abendroth G, Meshulam-Simon G, McCarty PL, Spormann AM. 2004. Molecular identification of the catabolic vinyl chloride reductase from *Dehalococcoides* sp. strain VS and its environmental distribution. Appl Environ Microbiol 70:4880–4888. https://doi.org/10.1128/AEM.70.8.4880-4888.2004.

14. Krajmalnik-Brown R, Hölscher T, Thomson IN, Saunders FM, Ritalahti KM, Löffler FE. 2004. Genetic identification of a putative vinyl chloride reductase in *Dehalococcoides* sp. strain BAV1. Appl Environ Microbiol 70:6347–6351. https://doi.org/10.1128/AEM.70.10.6347-6351.2004.

15. Löffler FE, Yan J, Ritalahti KM, Adrian L, Edwards EA, Konstantinidis KT, Müller JA, Fullerton H, Zinder SH, Spormann AM. 2013. *Dehalococcoides mccartyi* gen. nov., sp. nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation, belong to a novel bacterial class, *Dehalococcoidia* classis nov., order *Dehalococcoidales* ord. nov. and family *Dehalococcoidaceae* fam. nov., within the phylum *Chloroflexi*. Int J Syst Evol Microbiol 63:625–635. https://doi.org/10.1099/ijs.0.034926-0.

16. Zinder SH. 2016. The genus *Dehalococcoides*, p 107–136. *In* Adrian L, Löffler FE (ed), Organohalide-respiring bacteria. Springer, Berlin, Germany.

17. Yan J, Wang J, Villalobos Solis MI, Jin H, Chourey K, Li X, Yang Y, Yin Y, Hettich RL, Löffler FE. 2021. Respiratory vinyl chloride reductive dechlorination to ethene in TceA-expressing *Dehalococcoides mccartyi*. Environ Sci Technol 55:4831–4841. https://doi.org/10.1021/acs.est.0c07354.

18. He J, Sung Y, Krajmalnik-Brown R, Ritalahti KM, Löffler FE. 2005. Isolation and characterization of *Dehalococcoides* sp. strain FL2, a trichloroethene (TCE)- and 1,2-dichloroethene-respiring anaerobe. Environ Microbiol 7:1442–1450. https://doi.org/10.1111/j.1462-2920.2005.00830.x.

19. Maymó-Gatell X, Anguish T, Zinder SH. 1999. Reductive dechlorination of chlorinated ethenes and 1,2-dichloroethane by "Dehalococcoides ethenogenes" 195. Appl Environ Microbiol 65:3108–3113. https://doi.org/10.1128/AEM.65.7.3108-3113.1999.

20. Cupples AM, Spormann AM, McCarty PL. 2003. Growth of a *Dehalococcoides*-like microorganism on vinyl chloride and *cis*-dichloroethene as electron acceptors as determined by competitive PCR. Appl Environ Microbiol 69:953–959. https://doi.org/10.1128/AEM.69.2.953-959.2003.

21. Cupples AM, Spormann AM, McCarty PL. 2004. Vinyl chloride and *cis*-dichloroethene dechlorination kinetics and microorganism growth under substrate limiting conditions. Environ Sci Technol 38:1102–1107. https://doi.org/10.1021/es0348647.

22. Tang S, Chan WWM, Fletcher KE, Seifert J, Liang X, Löffler FE, Edwards EA, Adrian L. 2013. Functional characterization of reductive dehalogenases by using blue native polyacrylamide gel electrophoresis. Appl Environ Microbiol 79:974–981. https://doi.org/10.1128/AEM.01873-12.

23. Löffler FE, Edwards EA. 2006. Harnessing microbial activities for environmental cleanup. Curr Opin Biotechnol 17:274–284. https://doi.org/10.1016/j.copbio.2006.05.001.

24. Lee J, Im J, Kim U, Löffler FE. 2016. A data mining approach to predict in situ detoxification potential of chlorinated ethenes. Environ Sci Technol 50:5181–5188. https://doi.org/10.1021/acs.est.5b05090.

25. Clark K, Taggart DM, Baldwin BR, Ritalahti KM, Murdoch RW, Hatt JK, Löffler FE. 2018. Normalized quantitative PCR measurements as predictors for ethene formation at sites impacted with chlorinated ethenes. Environ Sci Technol 52:13410–13420. https://doi.org/10.1021/acs.est.8b04373.

26. Key TA, Richmond DP, Bowman KS, Cho Y-J, Chun J, da Costa MS, Rainey FA, Moe WM. 2016. Genome sequence of the organohalide-respiring *Dehalogenimonas alkenigignens* type strain (IP3-3^T). Stand Genomic Sci 11:44. https://doi.org/10.1186/s40793-016-0165-7.

27. Yan J, Rash BA, Rainey FA, Moe WM. 2009. Isolation of novel bacteria within the *Chloroflexi* capable of reductive dechlorination of 1,2,3-trichloropropane. Environ Microbiol 11:833–843. https://doi.org/10.1111/j.1462-2920.2008.01804.x.

28. Moe WM, Yan J, Nobre MF, da Costa MS, Rainey FA. 2009. *Dehalogenimonas lykanthroporepellens* gen. nov., sp. nov., a reductively dehalogenating bacterium isolated from chlorinated solvent-contaminated groundwater. Int J Syst Evol Microbiol 59:2692–2697. https://doi.org/10.1099/ijs.0.011502-0.

29. Maness AD, Bowman KS, Yan J, Rainey FA, Moe WM. 2012. *Dehalogenimonas* spp. can reductively dehalogenate high concentrations of 1,2-dichloroethane, 1,2-dichloropropane, and 1,1,2-trichloroethane. AMB Expr 2:54. https://doi.org/10.1186/2191-0855-2-54.

30. Bowman KS, Nobre MF, da Costa MS, Rainey FA, Moe WM. 2013. *Dehalogenimonas alkenigignens* sp. nov., a chlorinated-alkane-dehalogenating bacterium isolated from groundwater. Int J Syst Evol Microbiol 63:1492–1498. https://doi.org/10.1099/ijs.0.045054-0.

31. Key TA, Bowman KS, Lee I, Chun J, Albuquerque L, da Costa MS, Rainey FA, Moe WM. 2017. *Dehalogenimonas formicexedens* sp. nov., a chlorinated alkane-respiring bacterium isolated from contaminated groundwater. Int J Syst Evol Microbiol 67:1366–1373. https://doi.org/10.1099/ijsem.0.001819.

32. Trueba-Santiso A, Wasmund K, Soder-Walz JM, Marco-Urrea E, Adrian L. 2021. Genome sequence, proteome profile, and identification of a multiprotein reductive dehalogenase complex in *Dehalogenimonas alkenigignens* strain BRE15M. J Proteome Res 20:613–623. https://doi.org/10.1021/acs.jproteome.0c00569.

33. Ding C, Alvarez-Cohen L, He J. 2018. Growth of *Dehalococcoides mccartyi* species in an autotrophic consortium producing limited acetate. Biodegradation 29:487–498. https://doi.org/10.1007/s10532-018-9846-9.

34. Manchester MJ, Hug LA, Zarek M, Zila A, Edwards EA. 2012. Discovery of a *trans*-dichloroethene-respiring *Dehalogenimonas* species in the 1,1,2,2-tetrachloroethane-dechlorinating WBC-2 consortium. Appl Environ Microbiol 78:5280–5287. https://doi.org/10.1128/AEM.00384-12.

35. Molenda O, Quaile AT, Edwards EA. 2016. *Dehalogenimonas* sp. strain WBC-2 genome and identification of its *trans*-dichloroethene reductive dehalogenase, TdrA. Appl Environ Microbiol 82:40–50. https://doi.org/10.1128/AEM.02017-15.

36. Yang Y, Higgins SA, Yan J, Şimşir B, Chourey K, Iyer R, Hettich RL, Baldwin B, Ogles DM, Löffler FE. 2017. Grape pomace compost harbors organohalide-respiring *Dehalogenimonas* species with novel reductive dehalogenase genes. ISME J 11:2767–2780. https://doi.org/10.1038/ismej.2017.127.

37. Yang Y, Yan J, Li X, Lv Y, Cui Y, Kara-Murdoch F, Chen G, Löffler FE. 2020. Genome sequence of "*Candidatus* Dehalogenimonas etheniformans" strain GP, a vinyl chloride-respiring anaerobe. Microbiol Resour Announc 9:e01212-20. https://doi.org/10.1128/MRA.01212-20.

38. Gribble GW. 2015. A recent survey of naturally occurring organohalogen compounds. Environ Chem 12:396–405. https://doi.org/10.1071/EN15002.

39. Keppler F, Borchers R, Pracht J, Rheinberger S, Schöler HF. 2002. Natural formation of vinyl chloride in the terrestrial environment. Environ Sci Technol 36:2479–2483. https://doi.org/10.1021/es015611l.

40. Sexton DL, Chen G, Kara Murdoch F, Hashimi A, Löffler FE, Tocheva EI. 2022. Ultrastructure of organohalide-respiring *Dehalococcoidia* revealed by cryo-electron tomography. Appl Environ Microbiol 88: e01906-21. https://doi.org/10.1128/AEM.01906-21.

41. Moe WM, Rainey FA, Yan J. 2016. The genus *Dehalogenimonas*, p 137–151. *In* Adrian L, Löffler FE (ed), Organohalide-respiring bacteria. Springer, Berlin, Germany.

42. Sung Y, Ritalahti KM, Apkarian RP, Löffler FE. 2006. Quantitative PCR confirms purity of strain GT, a novel trichloroethene-to-ethene-respiring *Dehalococcoides* isolate. Appl Environ Microbiol 72:1980–1987. https://doi.org/10.1128/AEM.72.3.1980-1987.2006.

43. Lang AS, Zhaxybayeva O, Beatty JT. 2012. Gene transfer agents: phage-like elements of genetic exchange. Nat Rev Microbiol 10:472–482. https://doi.org/10.1038/nrmicro2802.

44. Zhuang W-Q, Yi S, Bill M, Brisson VL, Feng X, Men Y, Conrad ME, Tang YJ, Alvarez-Cohen L. 2014. Incomplete Wood-Ljungdahl pathway facilitates one-carbon metabolism in organohalide-respiring *Dehalococcoides mccartyi*. Proc Natl Acad Sci U S A 111:6419–6424. https://doi.org/10.1073/pnas.1321542111.

45. Chin BY, Otterbein LE. 2009. Carbon monoxide is a poison . . . to microbes! CO as a bactericidal molecule. Curr Opin Pharmacol 9:490–500. https://doi.org/10.1016/j.coph.2009.06.025.

46. Men Y, Feil H, VerBerkmoes NC, Shah MB, Johnson DR, Lee PKH, West KA, Zinder SH, Andersen GL, Alvarez-Cohen L. 2012. Sustainable syntrophic growth of *Dehalococcoides ethenogenes* strain 195 with *Desulfovibrio vulgaris Hildenborough* and *Methanobacterium congolense*: global transcriptomic and proteomic analyses. ISME J 6:410–421. https://doi.org/10.1038/ismej.2011.111.

47. Lee J, Lee TK, Löffler FE, Park J. 2011. Characterization of microbial community structure and population dynamics of tetrachloroethene-dechlorinating tidal mudflat communities. Biodegradation 22:687–698. https://doi.org/10.1007/s10532-010-9429-x.

48. Maphosa F, Lieten S, Dinkla I, Stams A, Smidt H, Fennell D. 2012. Ecogenomics of microbial communities in bioremediation of chlorinated contaminated sites. Front Microbiol 3:351. https://doi.org/10.3389/fmicb.2012.00351.

49. May HD, Miller GS, Kjellerup BV, Sowers KR. 2008. Dehalorespiration with polychlorinated biphenyls by an anaerobic ultramicrobacterium. Appl Environ Microbiol 74:2089–2094. https://doi.org/10.1128/AEM.01450-07.

50. Matturro B, Presta E, Rossetti S. 2016. Reductive dechlorination of tetrachloroethene in marine sediments: biodiversity and dehalorespiring capabilities of the indigenous microbes. Sci Total Environ 545–546:445–452. https://doi.org/10.1016/j.scitotenv.2015.12.098.

51. May HD, Sowers KR. 2016. "*Dehalobium chlorocoercia*" DF-1—from discovery to application, p 563–586. *In* Adrian L, Löffler FE (ed), Organohalide-respiring bacteria. Springer, Berlin, Germany.

52. U.S. Environmental Protection Agency. 2016. Average temperature of shallow ground water. https://www3.epa.gov/ceampubl/learn2model/part-two/onsite/ex/jne_henrys_map.html.

53. Friis AK, Heimann AC, Jakobsen R, Albrechtsen H-J, Cox E, Bjerg PL. 2007. Temperature dependence of anaerobic TCE-dechlorination in a highly enriched *Dehalococcoides*-containing culture. Water Res 41:355–364. https://doi.org/10.1016/j.watres.2006.09.026.

54. Marcet TF, Cápiro NL, Yang Y, Löffler FE, Pennell KD. 2018. Impacts of low-temperature thermal treatment on microbial detoxification of tetrachloroethene under continuous flow conditions. Water Res 145:21–29. https://doi.org/10.1016/j.watres.2018.07.076.

55. Bradley PM, Richmond S, Chapelle FH. 2005. Chloroethene biodegradation in sediments at 4°C. Appl Environ Microbiol 71:6414–6417. https://doi.org/10.1128/AEM.71.10.6414-6417.2005.

56. Yang Y, Cápiro NL, Marcet TF, Yan J, Pennell KD, Löffler FE. 2017. Organohalide respiration with chlorinated ethenes under low pH conditions. Environ Sci Technol 51:8579–8588. https://doi.org/10.1021/acs.est.7b01510.

57. Yang Y, Cápiro NL, Yan J, Marcet TF, Pennell KD, Löffler FE. 2017. Resilience and recovery of *Dehalococcoides mccartyi* following low pH exposure. FEMS Microbiol Ecol 93:fix130. https://doi.org/10.1093/femsec/fix130.

58. Pennell KD, Cápiro NL, Gaeth S, Marcet TF, Yang Y, Löffler FE. 2017. Secondary impacts of in situ remediation on groundwater quality and post-treatment management strategies: Strategic Environmental Research and Development Program (SERDP), SERDP project ER-2129, final report. Department of Defense, Washington, DC. https://www.serdp-estcp.org/content/download/47278/451071/file/ER-2129%20Final%20Report.pdf.

59. Löffler FE, Sanford RA, Ritalahti KM. 2005. Enrichment, cultivation, and detection of reductively dechlorinating bacteria. Methods Enzymol 397: 77–111. https://doi.org/10.1016/S0076-6879(05)97005-5.

60. Chen G, Kleindienst S, Griffiths DR, Mack EE, Seger ES, Löffler FE. 2017. Mutualistic interaction between dichloromethane- and chloromethane-degrading bacteria in an anaerobic mixed culture. Environ Microbiol 19: 4784–4796. https://doi.org/10.1111/1462-2920.13945.

61. Chen G, Fisch AR, Gibson CM, Mack EE, Seger ES, Campagna SR, Löffler FE. 2020. Mineralization versus fermentation: evidence for two distinct anaerobic bacterial degradation pathways for dichloromethane. ISME J 14: 959–970. https://doi.org/10.1038/s41396-019-0579-5.

62. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41: D590–D596. https://doi.org/10.1093/nar/gks1219.

63. Pruesse E, Peplies J, Glockner FO. 2012. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28: 1823–1829. https://doi.org/10.1093/bioinformatics/bts252.

64. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

65. Letunic I, Bork P. 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res 49:W293–W296. https://doi.org/10.1093/nar/gkab301.

66. Ritalahti KM, Amos BK, Sung Y, Wu Q, Koenigsberg SS, Löffler FE. 2006. Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple *Dehalococcoides* strains. Appl Environ Microbiol 72: 2765–2774. https://doi.org/10.1128/AEM.72.4.2765-2774.2006.

67. Hatt JK, Löffler FE. 2012. Quantitative real-time PCR (qPCR) detection chemistries affect enumeration of the *Dehalococcoides* 16S rRNA gene in groundwater. J Microbiol Methods 88:263–270. https://doi.org/10.1016/j.mimet.2011.12.005.

68. Hatt JK, Ritalahti KM, Ogles DM, Lebrón CA, Löffler FE. 2013. Design and application of an internal amplification control to improve *Dehalococcoides mccartyi* 16S rRNA gene enumeration by qPCR. Environ Sci Technol 47: 11131–11138. https://doi.org/10.1021/es4019817.

69. Kleindienst S, Higgins SA, Tsementzi D, Chen G, Konstantinidis KT, Mack EE, Löffler FE. 2017. "*Candidatus* Dichloromethanomonas elyunquensis" gen. nov., sp. nov., a dichloromethane-degrading anaerobe of the *Peptococcaceae* family. Syst Appl Microbiol 40:150–159. https://doi.org/10.1016/j.syapm.2016.12.001.

70. Yin Y, Yan J, Chen G, Murdoch FK, Pfisterer N, Löffler FE. 2019. Nitrous oxide is a potent inhibitor of bacterial reductive dechlorination. Environ Sci Technol 53:692–701. https://doi.org/10.1021/acs.est.8b05871.

71. Chen G, Jiang N, Villalobos Solis MI, Kara Murdoch F, Murdoch RW, Xie Y, Swift CM, Hettich RL, Löffler FE. 2021. Anaerobic microbial metabolism of dichloroacetate. mBio 12:e00537-21. https://doi.org/10.1128/mBio.00537-21.

72. Sander R. 2015. Compilation of Henry's law constants (version 4.0) for water as solvent. Atmos Chem Phys 15:4399–4981. https://doi.org/10.5194/acp-15-4399-2015.