

ORIGINAL ARTICLE

Grape pomace compost harbors organohalide-respiring *Dehalogenimonas* species with novel reductive dehalogenase genes

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Organohalide-respiring bacteria have key roles in the natural chlorine cycle; however, most of the current knowledge is based on cultures from contaminated environments. We demonstrate that grape pomace compost without prior exposure to chlorinated solvents harbors a *Dehalogenimonas* (*Dhgm*) species capable of using chlorinated ethenes, including the human carcinogen and common groundwater pollutant vinyl chloride (VC) as electron acceptors. Grape pomace microcosms and derived solid-free enrichment cultures were able to dechlorinate trichloroethene (TCE) to less chlorinated daughter products including ethene. 16S rRNA gene amplicon and qPCR analyses revealed a predominance of *Dhgm* sequences, but *Dehalococcoides mccartyi* (*Dhc*) biomarker genes were not detected. The enumeration of *Dhgm* 16S rRNA genes demonstrated VC-dependent growth, and $6.55 \pm 0.64 \times 10^8$ cells were measured per μmol of chloride released. Metagenome sequencing enabled the assembly of a *Dhgm* draft genome, and 52 putative reductive dehalogenase (RDase) genes were identified. Proteomic workflows identified a putative VC RDase with 49 and 56.1% amino acid similarity to the known VC RDases VcrA and BvcA, respectively. A survey of 1,173 groundwater samples collected from 111 chlorinated solvent-contaminated sites in the United States and Australia revealed that *Dhgm* 16S rRNA genes were frequently detected and outnumbered *Dhc* in 65% of the samples. *Dhgm* are likely greater contributors to reductive dechlorination of chlorinated solvents in contaminated aquifers than is currently recognized, and non-polluted environments represent sources of organohalide-respiring bacteria with novel RDase genes.

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Introduction

Chlorinated hydrocarbons have been widely used in different areas of modern societies, such as cleaning of machinery, manufacturing, and agrochemical production (for example, pesticides) (McCarty, 2010). Widespread usage and uncontrolled disposal of chlorinated hydrocarbons pose substantial environmental and human health concerns. For

example, the widely used chlorinated solvent trichloroethene (TCE) is carcinogenic (Wartenberg *et al.*, 2000) and has been implicated in Parkinson's disease (Goldman *et al.*, 2012). Vinyl chloride (VC), a TCE transformation product, is a notorious groundwater contaminant and a proven human carcinogen (Kielhorn *et al.*, 2000). TCE and VC are ranked #16 and #4 on the Substance Priority List (SPL) and have been detected in 1153 and 593 Superfund sites, respectively (www.atsdr.cdc.gov/spl/resources/index.html). The frequent detection of these hazardous chlorinated compounds in aquifers is disconcerting, and the discovery of organohalide-respiring bacteria was a landmark achievement, laying the foundation for *in situ* bioremediation (Löffler and Edwards, 2006). Diverse

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microorganisms, including members of the genera *Dehalobacter*, *Dehalococcoides*, *Desulfobacterium*, *Desulfuromonas*, *Geobacter* and *Sulfurospirillum* were isolated and demonstrated the ability to degrade PCE and TCE (Adrian and Löffler, 2016). Interestingly, the reductive dechlorination of chlorinated ethenes to non-toxic ethene has been attributed exclusively to *Dehalococcoides mccartyi* (*Dhc*) strains (Löffler *et al.*, 2013b), and a few reductive dehalogenase (RDase) genes have been implicated in the detoxification of chlorinated ethenes (Hug *et al.*, 2013b). Consequently, contaminated site characterization, bioremediation monitoring, and decision making rely on the quantitative assessment of *Dhc* biomarker genes in groundwater or aquifer solids (Cápiro *et al.*, 2014). Correlations between the presence and abundance of *Dhc* with the detoxification of chlorinated ethenes have been established; however, VC disappearance at sites lacking *Dhc* biomarkers has been observed (Lu *et al.*, 2006), and the presence/absence of *Dhc* biomarker genes does not always explain dechlorination activity and ethene formation (Da Silva and Alvarez, 2008; He *et al.*, 2015).

Information about microbial degradation of chlorinated ethenes has been almost exclusively derived from microorganisms obtained from contaminated environments. This approach was justified to derive process-relevant understanding for engineered bioremediation and site cleanup; however, more recent discoveries demonstrated that chlorinated hydrocarbons, including priority pollutants, also have natural origins (Gribble, 2010). For instance, even the human carcinogen VC can be generated abiotically in the soil environment, a process likely occurring since the first soils formed on Earth some 400 million years ago (Keppler *et al.*, 2002). Apparently, VC has been part of the biosphere long before human activities affected environmental concentrations of this carcinogen. A recent study correlated the abundance of *Dhc*-like *Chloroflexi* with the quantity of natural organohalogenes in soils, supporting the notion that the organohalide-respiring phenotype is not merely a consequence of anthropogenic activities (Krzmarzick *et al.*, 2012).

Here we demonstrate PCE reductive dechlorination and ethene formation in microcosms established with grape pomace compost not previously exposed to chlorinated solvents. Characterization of the enrichment culture demonstrated that the ability to grow with VC as electron acceptor is not limited to members of the *Dhc* genus. These findings expand the current understanding of the bacterial diversity capable of metabolizing VC under anoxic conditions, provide an explanation for ethene formation in chlorinated solvent-contaminated aquifers in the absence of *Dhc*, and demonstrate that ecosystems without prior chlorinated solvent exposure are a source of specialized organohalide-respiring bacteria, which use priority pollutants as electron acceptors.

Materials and methods

Chemicals

PCE, TCE and carbon tetrachloride (CT) (all $\geq 99\%$ purity) were purchased from Acros Organics (Distributed by VWR International, West Chester, PA, USA). *c*DCE ($> 96.0\%$ purity), VC ($\geq 99.5\%$), 1,2,3-trichloropropane (1,2,3-TCP) (99%), 1,2-dichloropropane (1,2-DCP) (99%), 1,2-dichloroethane (1,2-DCA) (99.8%), 1,1-dichloroethene (1,1-DCE) and ethene ($\geq 99.5\%$) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). All other chemicals were of scientific grade or better, and purchased from Sigma-Aldrich (St. Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA).

Microcosms and transfer cultures

Reduced, bicarbonate-buffered mineral salts medium with 5 mM lactate was prepared following established protocols (Löffler *et al.*, 2005). Microcosms were established with grape pomace compost collected in the wine-growing area of Rotenberg near Stuttgart, Germany (Supplementary Figure 1; Supplementary Information). Duplicate microcosms amended with 2 μ l neat tetrachloroethene (PCE) (19.5 μ mol/bottle) were incubated statically at room temperature in the dark. Subsequent transfers (3%, vol/vol) with lactate and PCE yielded solid-free, ethene-producing enrichment cultures. In subcultures, 2 ml VC (83.0 μ mol/bottle) replaced PCE as electron acceptor. To inhibit methanogenesis, 1.2 mM 2-bromoethanesulfonate (BES) was added. Live microcosms and transfer cultures were set up in at least duplicate. Negative controls included vessels that received no electron acceptor and autoclaved replicates of live incubations.

DNA extraction, PCR procedures and amplicon sequencing

DNA extraction and PCR assays targeting *Dhc* and *Dhgm* 16S rRNA genes and the *tceA*, *bvcA* and *vcrA* RDase genes followed established procedures (Ritalahti *et al.*, 2006; Yan *et al.*, 2009) and details are provided in the Supplementary Information. DNA extracted from PCE- and VC-fed culture GP (referring to the culture source, grape pomace) biomass was treated with the Genomic DNA Clean and Concentrator Kit (Zymo Research, Irvine, CA, USA). Purified DNA samples were PCR-amplified using barcoded-primers F515/R806 targeting the V4 region of the 16S rRNA gene. The amplicon sequencing approach followed established protocols (Caporaso *et al.*, 2011, 2012). Raw sequences were paired and analyzed using the mothur software package (www.mothur.org) following MiSeq standard operating procedures (Schloss *et al.*, 2009).

Quantitative real-time PCR assay

A TaqMan chemistry-based quantitative PCR (qPCR) assay was developed to specifically target and

enumerate *Dhgm* 16S rRNA genes. The forward primer Dhgm478F (5'-AGCAGCCGCGGTAATACG-3'), the reverse primer Dhgm536R (5'-CCACTTTA CGCCCAATAAATCC-3') and the TaqMan probe Dhgm500Probe (5'-6FAM-AGGCGAGCGTTAT-MGB-3') matching *Dhgm* 16S rRNA gene sequences, which were retrieved from NCBI database, were designed using the Primer3 plug-in in Geneious 8.1.7. The assay specificity was verified by *in silico* analysis using the Primer3 plug-in in Geneious 8.1.7 and the Primer-BLAST tool provided by NCBI, and was further experimentally confirmed using pure culture genomic DNA of *Dhc* strain 195 and strain FL2 and 171 bp-long synthesized DNA oligos containing the corresponding amplicon sequence of the *Dhc* 16S rRNA gene (Integrated DNA Technologies, Coralville, IA, USA) (Supplementary Figure 3). All qPCR assays consisted of two-fold concentrated TaqMan Universal PCR master mix (Applied Biosystems, Waltham, MA, USA), UltraPure nuclease-free water (Invitrogen, Carlsbad, CA, USA), 300 nM probe, 300 nM of each primer, and a DNA template and were performed using an Applied Biosystems ViiA 7 system (Applied Biosystems, Waltham, MA, USA). The qPCR cycle conditions were as follows: 50 °C for 2 min and then held at 95 °C for 10 min, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The standard curves were generated using three independent dilution series of plasmid DNA carrying a nearly complete sequence (1492 bp) of the *Dhgm* strain BL-DC-9 16S rRNA gene (NCBI accession NR_074337.1) or a 171 bp-long synthesized DNA oligo (Integrated DNA Technologies, Coralville, IA, USA) covering the 59 bp amplicon region. This *Dhgm* qPCR assay standard curve had a slope of -3.676, a y-intercept of 40.97, a R^2 value of 0.997, and a PCR amplification efficiency of 87.10% (Supplementary Figure 3). The detection limit is 11.4 gene copies per reaction, and the linear range spanned 1.14×10^2 to 1.14×10^8 gene copies per reaction.

Metagenome sequencing and comparative analyses

Metagenomic sequencing of the VC-grown enrichment culture GP was performed using the MiSeq platform provided through Center for Environmental Biotechnology (University of Tennessee Knoxville, Knoxville, TN, USA). The Nextera-prepped metagenome library with an average fragment size of 450 bp was sequenced using the v3 reagent kit for a 2x300 run. After being trimmed and filtered, the resulting 13 667 850 paired-end reads were assembled following established procedures (Oh *et al.*, 2011) (see Supplementary Information for details). For a comparative metagenome analysis, the sequences of VC-fed culture GP (MG-RAST ID: 4625853.3) were uploaded to the MG-RAST server and compared with three PCE-/TCE-dechlorinating consortia KB-1 (MG-RAST ID: 4450840.3), ANAS (MG-RAST ID: 4451655.3) and Donna II (MG-RAST

ID: 4451259.3). Metagenomic data sets from non-dechlorinating communities of an acid mine drainage site (Richmond Mine, Iron Mountain, CA; MG-RAST ID: 4441137.3 and 4441138.3) and a pristine freshwater lake in Antarctica (Ace Lake; MG-RAST ID: 4443683.3) were included in the analysis due to their well-documented meta information, good quality sequence reads, and their distinct origins. Taxonomic and functional annotation results from MG-RAST were imported into STAMP for principal component analysis (PCA) and visualization (Parks *et al.*, 2014). Details about the PCA analysis are provided in the Supplementary Information.

Genome binning and annotation

Binning of metagenomic contigs was conducted with MetaWatt v1.7 (Strous *et al.*, 2012) and VizBin (Laczny *et al.*, 2015), using GC content, tetranucleotide frequency and coverage as quality metrics to assess consistency of contigs within the genomic bin (Laczny *et al.*, 2015). Contigs belonging to the *Dhgm* bin were further assessed with CheckM (Parks *et al.*, 2015) using default settings to further assess genome bin completeness, contamination and taxonomic affiliation. The draft genome bin was uploaded to RAST (Overbeek *et al.*, 2014) for annotation (Accession ID: 1536648.4).

Phylogenetic analyses

Maximum likelihood tree estimation was performed using RAxML v8.2.5 (Stamatakis, 2014) on individual and concatenated 5S, 16S and 23S rRNA gene alignments with 1000 bootstrap replicates. Evolutionary model selection was evaluated with jModelTest v2.1.5 (Darriba *et al.*, 2012). In all cases, the generalized time-reversible model (Tavaré, 1986) with estimations of the proportion of invariable sites and rate heterogeneity among sites (eight substitution rate categories) was implemented in RAxML. For concatenated alignments, a partition file was provided to RAxML and rate heterogeneity among partitions was estimated individually. The phylogenetic tree of RDase proteins was built using Geneious software with default settings of the MAFFT Alignment and Geneious Tree Builder tools.

Proteomics analysis

Proteins were extracted from culture GP grown with TCE, 1,1-DCE, cDCE and VC as electron acceptors and subjected to LC-MS/MS analysis following established procedures (Chourey *et al.*, 2010, 2013). Biomass harvest times and detailed procedures are described in Supplementary Table 1 and the Supplementary Information.

Analytical methods

Chlorinated solvents were measured in 100 μ l headspace gas samples with an Agilent 7890A gas

chromatograph (GC) equipped with an Agilent DB624 column and a flame ionization detector (Agilent Technologies, Santa Clara, CA, USA). The concentrations of chlorinated ethenes were calculated by normalizing the peak areas to standard curves generated by adding known amounts of chlorinated ethenes and ethene into vessels with the same total volume and gas to liquid ratios. Gas samples (100 µl) were removed from the headspace using a gastight 250 µl Hamilton SampleLock syringe (Hamilton, Reno, NV, USA) and manually injected into the GC. Chloride release was calculated based on the gas chromatographic chlorinated ethene/ethene concentration measurements, and assumed that each reductive dechlorination step liberates one chlorine substituent as inorganic chloride (Sung et al., 2006).

Dhc and Dhgm abundances in groundwater impacted with chlorinated solvents

DNA was extracted from groundwater (1173 samples) or biomass collected on site onto Sterivex cartridges (Ritalahti et al., 2010). *Dhc* 16S rRNA gene-targeted qPCR followed established procedures (Ritalahti et al., 2006; Yan et al., 2009) and *Dhgm* 16S rRNA genes were enumerated with the Dhgm478F-Dhgm500Probe-Dhgm536R assay (this study).

Sequence data deposition

Raw metagenome sequences of the VC-dechlorinating culture GP were deposited on MG-RAST servers under accession ID 4625853.3. The draft genome of '*Candidatus* Dehalogenimonas etheniformans' strain GP was deposited to NCBI (accession number JQAN000000000 under BioProject SUB633157).

Results

Reductive dechlorination of chlorinated ethenes in grape pomace compost microcosms and transfer cultures

In anoxic grape pomace microcosms, PCE was reductively dechlorinated to ethene via TCE, cDCE, 1,1-DCE and VC as intermediates after a 300-day incubation period, and a similar dechlorination pattern was observed in transfer cultures (Supplementary Figure 2). Following the addition of BES, an inhibitor of methanogenesis, cDCE was the dechlorination end product and VC and ethene were not formed (data not shown). Without BES addition, the solid-free transfer cultures maintained the ability to produce ethene in defined, completely synthetic medium.

Comparison between PCE- and VC-fed enrichment cultures

To identify the population(s) responsible for the observed dechlorination activity, DNA was extracted

from ethene-producing PCE- and VC-fed cultures for 16S rRNA gene amplicon profiling. No evidence for the presence of *Dhc* 16S rRNA gene sequences was obtained, a finding supported by targeted PCR analyses, which failed to detect the *Dhc* 16S rRNA gene and the *tceA*, *bvcA*, and *vcrA* RDase genes implicated in reductive dechlorination of chlorinated ethenes (data not shown). Instead, *Dhgm* 16S rRNA gene fragments dominated the sequence pool, and represented 43.9 and 46.1% of all bacterial sequences in the PCE-fed and in VC-fed cultures, respectively (Figure 1). Also detected were sequences of not-yet-cultured bacteria of the WWE1 and WPS-2 candidate divisions, which represented 3.6% and 8.3% of the PCE-fed and 2.6% and 9.4% of the VC-fed communities, respectively. Sequences assigned to the genera *Clostridium*, *Pelotomaculum*, *Treponema*, *Sedimentibacter*, as well as the unclassified groups HA73 of Dethiosulfovibrionaceae and vadinCA02 of Synergistaceae, were all present at >1% abundances in both PCE- and VC-fed communities.

Growth of Dhgm coupled with VC-to-ethene reductive dechlorination

In solid-free enrichment cultures, VC dechlorination to ethene commenced after a lag phase of about 20 days and stoichiometric amounts of ethene were

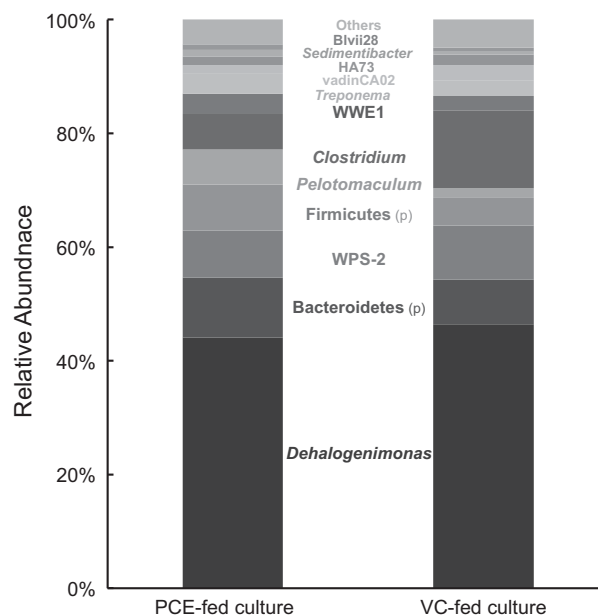


Figure 1 Community structure of PCE-fed and VC-fed grape pomace enrichment cultures as revealed by 16S rRNA gene amplicon sequencing. The relative abundances of operational taxonomic units (OTUs) representing bacteria are shown at the genus level. Rare groups representing <1% of total bacterial community were categorized as 'Others'. OTUs representing the phyla Bacteroidetes and Firmicutes could not be classified at the genus level. WPS-2, WWE1, vadinCA02 and HA73 represent uncultured bacterial groups. The full colour version of this figure is available at *ISME* Journal online.

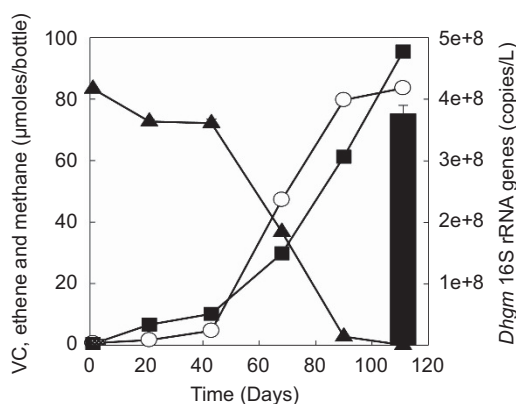


Figure 2 VC-to-ethene reductive dechlorination in the enrichment culture harboring strain GP (triangles, VC; squares, methane; open circles, ethene; solid bar, *Dhgm* 16S rRNA gene copies). Data points are averages of duplicate cultures and the error bars show one standard deviation. If no error bars are shown, the standard deviations were too small to be illustrated.

produced over a 50-day incubation period (Figure 2). qPCR results demonstrated that the *Dhgm* 16S rRNA gene copies per mL increased from $1.02 \pm 0.12 \times 10^7$ (cells transferred with the inoculum) to $3.76 \pm 0.14 \times 10^8$ (a 37-fold increase) following complete VC degradation. The growth yields of the *Dhgm* population in culture GP were in the range reported for VC-dechlorinating *Dhc* strains and up to 2 orders of magnitude higher than those reported for *Dhgm lykanthroporepellens* strain BL-DC-9 grown with chlorinated propanes (Supplementary Table 2). Following seven repeated transfers to fresh medium with VC as electron acceptor, culture GP maintained the ability to dechlorinate TCE, 1,1-DCE and *c*DCE to ethene (Supplementary Figure 4), and $1.66 \pm 0.44 \times 10^9$, $7.21 \pm 0.45 \times 10^8$ and $1.72 \pm 0.01 \times 10^9$ cells per μmol chlorinated electron acceptor consumed were produced. The VC-enriched culture GP lost the ability to dechlorinate PCE, and other potential chlorinated electron acceptors including CT, 1,2-DCA, 1,2,3-TCP and 1,2-DCP were not dechlorinated (data not shown).

Metagenome analyses

A comparative analysis of taxonomic ranks derived from the metagenomic sequences of culture GP, the *Dhc*-containing consortia ANAS, KB-1, Donna II and two non-dechlorinating microbial communities revealed that the metagenomes from dechlorinating and non-dechlorinating cultures did not produce distinct clusters (Figure 3a). Instead, the metagenomes were dispersed and the first principal component explained more than three times the variation observed between communities containing dechlorinating and non-dechlorinating members than principal component two (Figure 3a). The separation between samples observed along the first principal component axis can be attributed to differences in the relative abundances of taxonomic ranks

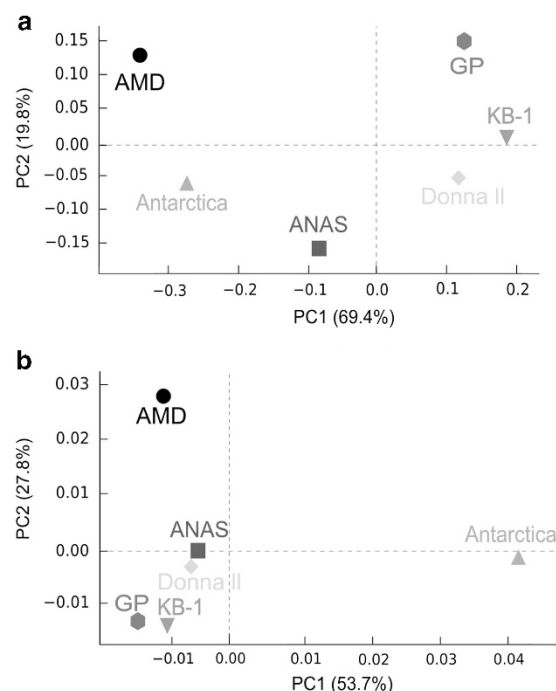


Figure 3 Principal component analysis of taxonomic profiles at the phylum level (a) and functional profiles (b) of metagenomes from the dechlorinating consortia ANAS, Donna II, KB-1 and GP. Included in the analysis were the metagenome data sets from two non-dechlorinating microbial communities (AMD and Antarctica). For the functional comparison (b), the metagenomic sequences were classified into SEED categories and the distribution of SEED categories was compared. The full colour version of this figure is available at *ISME* Journal online.

representing the phyla Chloroflexi, Proteobacteria, Actinobacteria and Cyanobacteria among metagenomes containing dechlorinating and non-dechlorinating taxa (Supplementary Table 3). For example, the average relative abundance of sequence reads representing Proteobacteria within non-dechlorinating metagenomes was $37.4 \pm 9.90\%$, but were lower ($13.6 \pm 4.90\%$) in the metagenomes of the dechlorinating consortia. Sequences derived from members of the Chloroflexi had higher relative abundances in dechlorinating communities, whereas sequences representing the phyla Actinobacteria and Cyanobacteria had higher average relative abundances in metagenomes derived from the non-dechlorinating communities (Supplementary Table 3). In contrast, an ordination of the relative abundances of functional categories detected in the metagenomes indicated that the dechlorinating communities were more similar to each other than to the non-dechlorinating communities (Figure 3b). Specifically, the relative abundance of sequences assigned to branched-chain amino acid biosynthesis ($0.17 \pm 0.05\%$ in dechlorinating versus $0.06 \pm 0.02\%$ in non-dechlorinating metagenomes), formate metabolism (0.11 ± 0.01 versus 0.00%), iron transport (0.15 ± 0.04 versus $0.02 \pm 0.03\%$), and reductive dechlorination (0.76 ± 0.44 versus 0.00%) were enriched in the metagenomes of dechlorinating

consortia (Supplementary Table 4). Further efforts to characterize the VC-dechlorinating culture GP relied on assigning genes from the assembled metagenome to the SEED (www.theseed.org) functional groups. More than 50% of the coding sequences from assembled contigs could not be assigned to a SEED functional category, indicating the presence of many genes with unknown functions in the VC-dechlorinating mixed culture GP. Among assigned SEED functional categories, genes encoding the metabolism of carbohydrates, amino acids and derivatives, proteins, DNA and cofactors/vitamins/prosthetic groups/pigments were highly represented in the assembled reads (Supplementary Table 5).

Draft genome of the VC-dechlorinating *Dhgm* population

Binning of the metagenome sequences allowed the assembly of 16 contigs ranging in size between 1.0 kbp and 666.8 kbp (N50 = 277.2 kbp), and a draft genome of the organohalide-respiring *Dhgm* population was obtained. The draft genome had a size of 2.02 Mbp with a G+C content of 52%. CheckM analysis indicated that the genome was 94% complete (144 single copy marker genes detected) with 0% contamination and no strain heterogeneity (default 90% amino acid identity cutoff). Average contig coverage ranged between 7.5 and 505-fold, with an average genome coverage of 276-fold. Prokka annotation of the genomic bin predicted a total of 2099 genes including three ribosomal RNAs (5S, 16S and 23S rRNA), 2036 coding DNA sequences (CDS), 14 non-coding RNA sequences and 46 transfer RNAs. Pairwise sequence comparisons demonstrated that the 16S rRNA gene sequence representing the *Dhgm*

sp. strain GP shares 99.3%, 96.9%, 96.0% and 95.3% sequence identities with *Dhgm formicexedens* strain NSZ-14 (Key et al., 2017), *Dhgm alkenigignens* strain IP3-3 (Key et al., 2016), *Dhgm* sp. strain WBC-2 (Molenda et al., 2016), and *Dhgm lykanthroporepellens* strain BL-DC-9 (Moe et al., 2009), respectively. Phylogenetic analysis based on concatenated 5S-16S-23S rRNA gene alignments supported *Dhgm* sp. strain GP's affiliation with the *Dhgm* genus (Figure 4). A total of 2021 orthologous gene clusters were shared between the genomes of *Dhgm* strains NSZ-14, IP3-3, WBC-2, BL-DC-9 and GP and the genome of *Dhc* strain 195, and 1,921 gene orthologous clusters were shared by at least two genomes. *Dhgm* sp. strain GP shared 1454, 1294, 1159, 1047 and 915 orthologous clusters with *Dhgm* strains NSZ-14, IP3-3, WBC-2, BL-DC-9 and *Dhc* strain 195, respectively (Supplementary Figure 5). Although *Dhgm* sp. strain GP shares 99.3% 16S rRNA gene sequence similarity with strain NSZ-14, the genome-aggregate average nucleotide identity (ANI) between both organisms is only 80.59%, well below the 95% ANI that corresponds to the 70% DNA–DNA hybridization standard that is frequently used for species demarcation (enve-omics.ce.gatech.edu; Löffler et al., 2013b), suggesting strain GP represents a distinct species.

A characteristic feature of obligate organohalide-respiring bacteria is the presence of multiple hydrogenase genes. Gene clusters encoding a [Ni/Fe] hydrogenase complex (EC 1.12.2.1), a NAD-reducing hydrogenase complex (EC 1.12.1.2), a periplasmic [Fe] hydrogenase complex (EC 1.12.7.2) and an uptake hydrogenase complex (EC 1.12.99.6) were identified on the draft genome. Similar to the other three sequenced *Dhgm* genomes (that is, strains

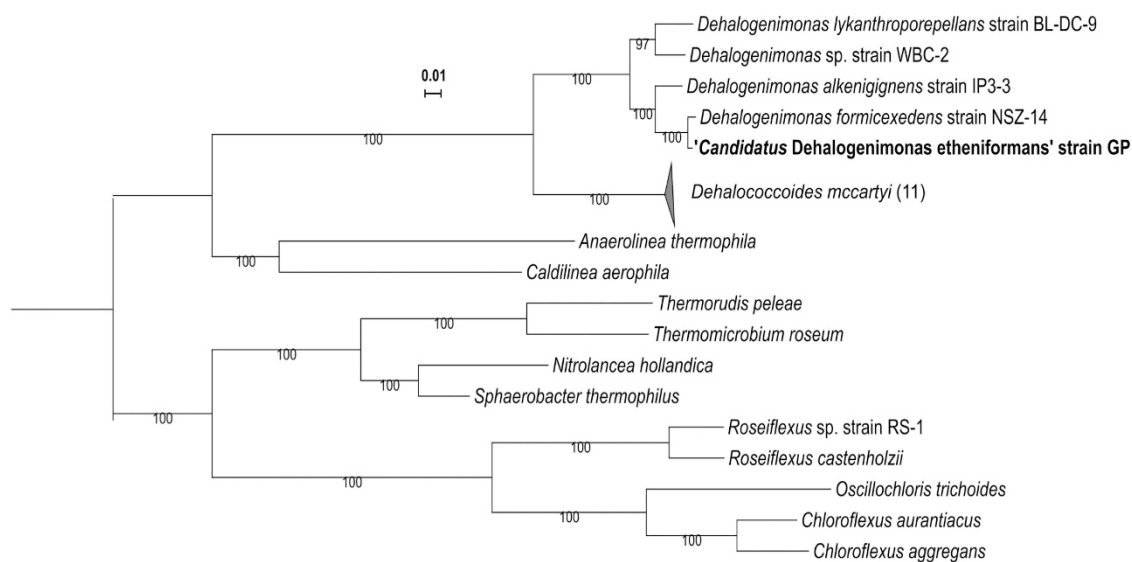


Figure 4 Midpoint rooted maximum likelihood phylogenetic tree showing the relationship of ‘*Candidatus Dehalogenimonas etheniformans*’ to other members of the Chloroflexi based on concatenated 5S-16S-23S rRNA genes. The closest relatives of ‘*Candidatus Dehalogenimonas etheniformans*’ were *Dhgm formicexedens* strain NST-14^T and *Dhgm alkenigignens* strain IP3-3. The accession numbers for each genome are listed in Supplementary Table 8. The scale bar indicates 0.01 nucleotide substitution per site.

BL-DC-9, IP3-3 and NSZ-14), which possess multiple genes annotated as formate dehydrogenase alpha subunit (Fdh), two genes encoding Fdh (EC 1.2.1.2) were identified on the genome of strain GP, whereas *Dhc* genomes harbor only one copy of the *fdh* gene. The phylogenetic analysis of putative *Dhgm* and *Dhc* Fdh proteins suggested that *Dhgm* may possess two types: one related to the *Dhc*-type Fdh (53.3% amino acid identity), while the other is more similar to *Clostridium* Fdh proteins (up to 48.7% identity) (Supplementary Figure 6). The *Dhc*-type Fdh has cysteine at the putative active site, while *Dhgm*-type Fdh has different amino acids (for example, cysteine or selenocysteine) at the same site (Supplementary Figure 7). A total of 52 putative RDase genes were identified, 10 of which were associated with B genes that encode B proteins with 1 to 4 transmembrane spanning helices (Supplementary Table 6). All putative RDase protein sequences had either a TAT signal peptide or a Sec signal peptide predicted by PRED-TAT (Supplementary Table 6) (Bagos *et al.*, 2010). One of the predicted RDases (prokka_01475) shared 36.8%, 67.6% and 65.8% identities with the three characterized PcbA RDases identified in *Dhc* strains CG1, CG4 and CG5, respectively (Wang *et al.*, 2014).

Although genes encoding *c*-type cytochromes were not found, two *c*-type cytochrome biogenesis genes, *ccsA* and *ccsB* (Hamel *et al.*, 2003), were present in the strain GP draft genome. Both *c*-type cytochrome biogenesis genes were also present in other sequenced *Dhgm* and *Dhc* genomes but their functions remain unclear. Genes for *de novo* corrin ring biosynthesis were absent, but genes implicated in corrinoid salvage and modification (that is, *cobA*, *cbiP*, *cbiB*, *cobU*, *cobT*, *cobC*, *cobS* and *cbiZ*) were detected. Similar to observations made with *Dhc*, the *Dhgm* genome possesses genes encoding two distinct cobinamide (Cbi)-salvaging pathways: the bacterial pathway relying on *cobU/cobP* genes and the archaeal *cbiZ* pathway. Genes coding for the vitamin B₁₂ ABC transporter *BtuFCD* and the dual-function cobalt/nickel transporter system *cbiMNQO* were also identified.

The function of a putative heterodisulfide reductase gene cluster (*hdrABC*) in the genome of *Dhgm* strain GP is unclear but may be involved in electron bifurcation systems (for example, HdrABC-MvhADG of *Methanothermobacter marburgensis*, HdrABC-FlxABCD of *Desulfovibrio vulgaris* Hildenborough). In the electron bifurcation system of *Desulfovibrio vulgaris* Hildenborough, the HdrABC complex is responsible for transferring electrons (released from hydrogen or NADH+H⁺ oxidation catalyzed by the FlxABCD complex) to oxidized ferredoxin and a second electron acceptor small protein (for example, DsrC) (Ramos *et al.*, 2015). While orthologs of genes encoding HdrABC were observed across all available *Dhgm* genomes, none were detected in any sequenced *Dhc* genome, suggesting *Dhgm* and *Dhc* may employ different electron transfer systems. It is

also worth mentioning that the *Dhgm* draft genome harbors two genes in a single operon, which encode proteins sharing greater than 39 and 54% amino acid identity to *arsD* and *arc3* of *Escherichia coli*, suggesting *Dhgm* strain GP experienced arsenic selection pressure and is capable of detoxifying arsenicals.

Protein profiling and identification of a novel putative VC RDase

Dhgm strain GP grew with VC as electron acceptor, but qPCR assays failed to detect the known VC RDase genes *tceA*, *vcrA* and *bvcA*, suggesting strain GP harbors a different (novel) VC RDase. The proteomics analysis identified three abundant putative RDases, prokka_00862, prokka_01300, and prokka_02004, in GP cultures grown with TCE, 1,1-DCE and VC, respectively (Figure 5; Supplementary Table 7). In *c*DCE-grown cultures, prokka_00862 and prokka_02004 had similar measured abundances (that is, normalized spectral counts), suggesting equal expression. The abundances of the detected peptides indicated high expression of the prokka_02004 RDase in cells collected from VC-grown GP cultures, while a lower abundance of the prokka_02004 RDase was observed in TCE-, 1,1-DCE-, *c*DCE-grown GP cultures (Supplementary Table 7). The prokka_02004 abundance correlated with the amount of ethene formed in the cultivation vessels amended with different chlorinated ethenes, supporting the proposition that prokka_02004 has activity towards VC. Prokka_02004 grouped in a cluster comprising the characterized RDases TceA, BvcA and VcrA (Figure 6), and shared 83.3, 56.1 and 49% amino acid similarity (76.2, 42.1 and 34.9% amino acid identity) with the TceA, and the characterized VC RDases BvcA and VcrA, respectively. No peptides representing RDase B proteins encoded in the genome of *Dhgm* strain GP were detected. Based on observations from both the phylogenetic (Figure 6) and proteomics analysis (Figure 5, Supplementary Table 7), the prokka_02004 RDase likely encodes a VC RDase and was designated *cerA* (that is, chloroethene reductase gene). Further examination of additional proteins detected in the *Dhgm* strain GP during active dechlorination of different chlorinated

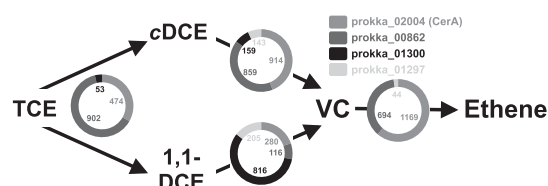


Figure 5 Relative RDase A protein abundances based on normalized spectral counts of peptides detected in cultures grown with TCE, *c*DCE, 1,1-DCE and VC as electron acceptors. The numbers indicate the normalized spectral counts. The full colour version of this figure is available at *ISME Journal* online.

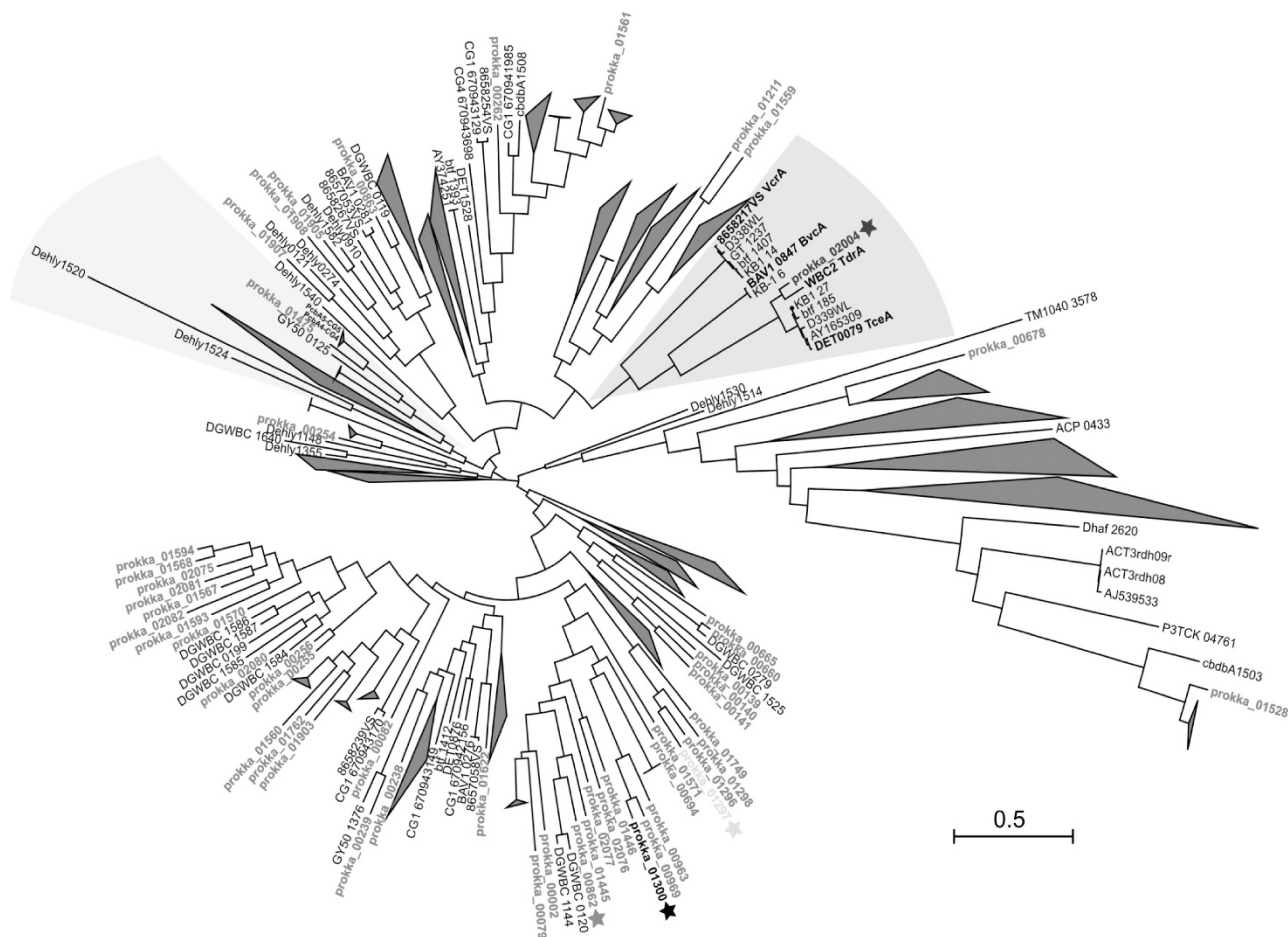


Figure 6 Phylogenetic relationships of 528 RDase A protein sequences. The analysis included 355 sequences reported by Hug et al. (Hug and Edwards, 2013) plus the RDase sequences of *Dhc* strains CG1, CG4, CG5, SG1, and *Dhgm* strains WBC-2 and GP (Supplementary Information). Shaded in green and blue are clusters comprising known PCB and VC RDases, respectively. Putative RDases of strain GP are shown in red font and the stars highlight RDases prokka_02004 (blue), prokka_01300 (black), prokka_01297 (green) and prokka_00862 (red). The scale bar indicates 0.5 amino acid substitution per site. The full colour version of this figure is available at *ISME* Journal online.

ethenes revealed an abundance of chaperonin proteins (GroES, GroEL and Hsp20) (Supplementary Table 7). High levels of rubrerythrin, thioredoxin and two proteins annotated as formate dehydrogenases (prokka_01480 and prokka_01481) were also observed.

Detection and abundance of *Dhgm* at sites impacted with chlorinated solvents

For contaminated site assessment, monitoring, and remediation treatment decision making, the quantitative measurement of *Dhc* biomarker genes has become routine practice. In 1173 groundwater samples collected from 111 chlorinated solvent-impacted sites, 16S rRNA genes of both *Dhc* and *Dhgm*, *Dhc* only and *Dhgm* only were detected in 849, 79 and 97 samples, respectively. In 148 samples (<13%), neither *Dhgm* nor *Dhc* 16S rRNA genes were detected. In 812 samples with quantifiable *Dhgm* and *Dhc* cell numbers, *Dhgm* outnumbered *Dhc* in the majority (65%) of samples and the *Dhgm*/*Dhc* ratio was above 1 in 524 samples (Figure 7).

These findings indicate that *Dhgm* populations contribute more to the reductive dechlorination process in contaminated aquifers than has been previously acknowledged.

Description of ‘*Candidatus Dehalogenimonas etheniformans*’

‘*Candidatus Dehalogenimonas etheniformans*’ (*e.the.ni.for'mans*. N.L. n. *ethenum*, ethene; L. part. adj. *formans* forming; N.L. part. adj. *etheniformans* ethene forming). The species designation reflects the organism’s ability to form ethene from chlorinated ethenes. ‘*Candidatus Dehalogenimonas etheniformans*’ utilizes TCE, *c*DCE, 1,1-DCE and VC as respiratory electron acceptors. ‘*Candidatus Dehalogenimonas etheniformans*’ can be cultivated in mixed culture using hydrogen as electron donor, and acetate and/or lactate as carbon sources in bicarbonate-buffered medium. Growth occurs at 20–30 °C and circumneutral pH. The G+C content of strain GP is 52 mol%. Strain GP was identified in a mixed culture derived from non-contaminated grape

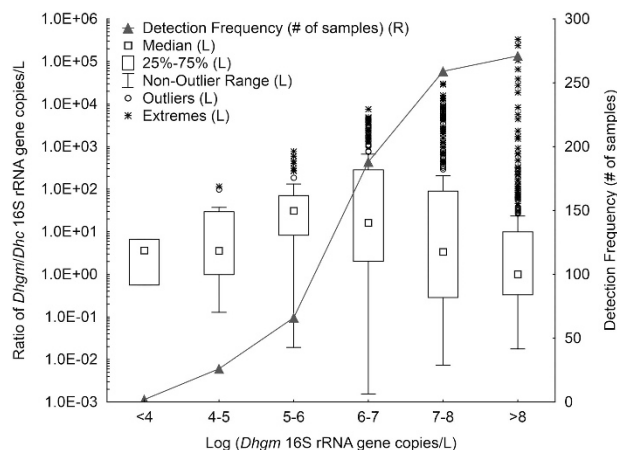


Figure 7 Distribution of *Dhgm* and *Dhc* in 1173 groundwater samples collected from 111 chlorinated solvent-impacted sites. Boxes represent the upper (75th) and lower (25th) quartiles and whiskers depict the non-outlier range. Outliers have *Dhgm/Dhc* ratios greater than the upper quartile *Dhgm/Dhc* ratio plus 1.5 times the difference between upper and lower quartile *Dhgm/Dhc* ratios, or less than the lower quartile *Dhgm/Dhc* ratio minus 1.5 times the difference between upper and lower quartile *Dhgm/Dhc* ratios. *Dhgm/Dhc* ratios greater than the upper quartile plus 2 times the difference of the quartiles or less than the lower quartile minus 2 times the difference between the quartiles were defined as extremes. Box plots were created using Statistica v12.0 (StatSoft, Inc., Tulsa, OK, USA). The full colour version of this figure is available at ISME Journal online.

pomace compost collected from the wine-growing region of Rotenberg near Stuttgart, Germany (48° 46' 58.0" N 9° 17' 14.8" E). Phylogenetic, genotypic and phenotypic characteristics place strain GP in the *Dhgm* genus within the organohalide-respiring Chloroflexi, and we propose that strain GP represents a new species, '*Candidatus Dehalogenimonas etheniformans*'.

Discussion

Among the organohalide-respiring Chloroflexi, *Dhc* have received most attention because of their ability to detoxify priority pollutants (He *et al.*, 2003; Wang *et al.*, 2014), their demonstrated relevance for *in situ* bioremediation (Löffler and Edwards, 2006; Stroo *et al.*, 2012), and the availability of representative isolates (Maymó-Gatell *et al.*, 1997; Adrian *et al.*, 2000; He *et al.*, 2003) and bioaugmentation consortia (Löffler *et al.*, 2013a; Adrian and Löffler, 2016). To date, metabolic VC-to-ethene reductive dechlorination has been exclusively linked to the presence and activity of *Dhc* strains, and the value of monitoring *Dhc* 16S rRNA genes and the *Dhc* RDase genes *tceA*, *vcrA* and *bvcA* for supporting contaminated site management decisions has been demonstrated (Stroo *et al.*, 2012). At sites, where VC disappearance was observed but *Dhc* were not detected, VC degradation was attributed to other processes, including abiotic reactions mediated by mineral phases such as magnetite (He *et al.*, 2015) or aerobic microbial VC oxidation (Coleman *et al.*, 2002; Mattes *et al.*, 2010).

The discovery of non-*Dhc* populations carrying novel VC RDase genes indicates that a broader diversity of microorganisms contributes to anaerobic VC metabolism and detoxification. This relevant observation demonstrates that the absence of known *Dhc* biomarker genes should not be used as an argument that the microbial reductive dechlorination process is not driving contaminant removal. A survey of 1173 groundwater wells from chlorinated solvent-impacted sites demonstrated that *Dhgm* were similarly distributed as *Dhc*, and in fact outnumbered *Dhc* in the majority of the wells where both *Dhc* and *Dhgm* were detected. Considering that a number of these samples were influenced by bioaugmentation with *Dhc*- (but not *Dhgm*-) containing consortia, *Dhgm* likely outnumber *Dhc* in even more of the sampling locations prior to bioremediation treatment. The known *Dhgm* genomes indicate a strict organohalide-respiring energy metabolism, and it is very likely that the presence of *Dhgm* implies that these bacteria contribute to *in situ* reductive dechlorination. Thus, the contribution of this organismal group to attenuation of chlorinated solvent contaminants is probably far greater than is currently appreciated.

Enrichment efforts with chlorinated ethenes from contaminated aquifer materials generally yield *Dhc*- rather than *Dhgm*-containing cultures. A possible reason is the slower growth of *Dhgm* (Supplementary Table 2) and *Dhc* may outcompete *Dhgm* strains in laboratory enrichment cultures. Very likely, refined growth conditions (for example, medium composition) will better meet the nutritional requirements of chlorinated ethene-dechlorinating *Dhgm*, an issue that has also limited the initial experimental efforts with *Dhc* cultures (Löffler *et al.*, 2013b). For example, it was recently demonstrated that the lower base of the essential RDase corrinoid prosthetic group can affect reductive dechlorination rates and products (Yan *et al.*, 2016), and the exact cobamide requirement to support efficient CerA maturation and catalytic activity has yet to be elucidated.

The majority of *Dhc* and *Dhgm* isolates and mixed cultures were derived from contaminated environments, with the exception of *Dhc* strain FL2, which was isolated from river sediment without reported chlorinated solvent exposure (He *et al.*, 2005). Culture GP containing the first member of the *Dhgm* genus capable of using vinyl chloride as respiratory electron acceptor was obtained from grape pomace compost that had no prior exposure to chlorinated solvents. An earlier study found several Dehalococcoidia-type 16S rRNA gene sequences associated with a fluidized bed reactor digesting wine distillation waste (Godon *et al.*, 1997), suggesting that organohalide-respiring Chloroflexi are members of the microbiome associated with processed grapes. This raises the question as to why an organism whose energy metabolism hinges on the presence of certain organohalogenes, including chlorinated ethenes, is found in grape pomace that has never

encountered chlorinated solvents? A plausible explanation is the active production of organohalogenes in soils including composts, which support organohalide-respiring Chloroflexi such as strain GP. An unprecedented number of 52 putative RDase genes were identified on strain GP's genome, indicating a possible adaptation to the non-contaminated environment from where the culture was obtained. For survival in pristine environments, obligate organohalide-respiring bacteria must rely on naturally produced organohalogenes, and very likely use a diversity of halogenated compounds to derive sufficient energy for cell maintenance and growth. Evidence is accumulating that chlorinated hydrocarbons, including priority pollutants such as VC, are produced naturally in many environments, including soils (Keppler *et al.*, 2002; Gribble, 2010). This is an important observation suggesting that RDases that use priority pollutants (for example, VC) as substrates evolve in environments without anthropogenic chlorinated solvent contamination. A survey detected 16S rRNA gene fragments of *Dhc*-like Chloroflexi in nearly 90% of the investigated 116 soil samples collected from locations not impacted by anthropogenic chlorinated hydrocarbons (Krzmarzick *et al.*, 2012). A 16S rRNA gene sequencing effort identified *Dhgm* sequences at 60 m depth in a perennially stratified, pristine Arctic lake with saline, anoxic bottom waters (Comeau *et al.*, 2012). *Dhgm* 16S rRNA gene sequences were also discovered in the photic layer (0–25 m) and the near-bottom zone (1465–1515 m) of Lake Baikal, the world's deepest and most voluminous lake (Kurilkina *et al.*, 2016). Recent work identified Dehalococcoidia-type sequences in a permanently ice-coved lake in Antarctica, and the authors speculated that hydrogenotrophic reductive dehalogenation could fuel anaerobic methane oxidation (Saxton *et al.*, 2016). Consistent with the hypothesis that organohalide-respiring bacteria evolved long before human activities released chlorinated chemicals into the environment, our findings provide additional support that pristine environments harbor specialized organohalide-respiring bacteria that use chlorinated hydrocarbons, including chlorinated ethenes, as electron acceptors. It is plausible that this bacterial respiration evolved in response to soil processes generating organohalogenes, which could have started when soils first formed in the late Silurian to Early Devonian some 400 million years ago (Keppler *et al.*, 2000, 2002; Fahimi *et al.*, 2003). Quite possibly, the natural formation of organohalogenes dates back even further in marine systems (Gribble, 2010).

Obligate organohalide-respiring *Dhgm* and *Dhc* characteristically encode multiple RDase genes on their genomes. For example, 19, 32, 36, 11, 19, 22 RDase genes were identified on the genomes of *Dhc* strains 195, CBDB1, VS, BAV1, *Dhgm* strain BL-DC-9 and strain WBC-2, respectively (Adrian and Löffler, 2016), suggesting that the utilization of a broader suite of organohalogenes as electron acceptors is a

common feature. *Dhgm* strain GP possesses 52 putative RDase genes and the RDases prokka_01300, prokka_01297, prokka_00862, and prokka_02004 (CerA) were expressed in cultures grown with chlorinated ethenes. Proteomics analysis revealed that prokka_01300 was highly expressed when culture GP was grown with 1,1-DCE, suggesting this RDase is involved in 1,1-DCE reductive dechlorination. The putative RDase prokka_01297 was detected in GP cultures grown with *c*DCE, 1,1-DCE and VC, albeit with significantly lower spectral counts than the major RDases. Previous studies revealed the expression of multiple RDases in organohalide-respiring Chloroflexi grown with polychlorinated benzenes as electron acceptors, a phenomenon that is currently not understood (Schiffmann *et al.*, 2014; Pöritz *et al.*, 2015). The putative RDase prokka_00862 was differentially expressed when culture GP was cultivated with TCE, 1,1-DCE, *c*DCE and VC (Figure 5). Phylogenetic analysis did not group prokka_00862 with the known VC RDases, but affiliated this protein with the putative RDase DGWBC_1144 identified in *Dhgm* sp. strain WBC-2 (Figure 6). *Dhgm* sp. strain WBC-2 is responsible for transforming *trans*-1,2-dichloroethene (*t*DCE) to VC in a mixed culture enriched with 1,1,2,2-tetrachloroethane (Molenda *et al.*, 2016). During growth with *t*DCE, strain WBC-2 expressed four RDases (DGWBC_0411, DGWBC_1144, DGWBC_1574 and DGWBC_0279), and blue native polyacrylamide gel electrophoresis (BN-PAGE) analysis identified DGWBC_0411, designated TdrA, as the *t*DCE-to-VC RDase (Molenda *et al.*, 2016). No specific activity could be assigned to DGWBC_1144 but strain GP expressed a related protein (prokka_00862), and these putative RDases may have functional roles in reductive dechlorination of chlorinated ethenes. Strain GP also possesses an RDase (prokka_01475) that shares more than 65% amino acid identity with the PCB RDases PcbA-CG4 and PcbA-CG5 of *Dhc* strains CG4 and CG5, respectively (Figure 6). *Dhgm* 16S rRNA gene sequences have been detected in PCB-dechlorinating enrichment cultures (Wang and He, 2013) and PCB-impacted marine sediment (Klaus *et al.*, 2016), suggesting that prokka_01475 represents a potentially novel PCB RDase.

Characterization of *Dhc* genomes showed that the majority of RDase A genes encoding the catalytically active RDases are associated with RDase B genes encoding putative membrane-anchor proteins (for example, 32 out of 32, 8 of 11, and 29 of 38 putative RDase A genes possess accompanying B genes in *Dhc* strain CBDB1, strain 11a, and strain MB, respectively) (Kube *et al.*, 2005; Low *et al.*, 2015). By comparison, *Dhgm lykanthroporepellens* strain BL-DC-9 possesses 17 putative RDase A genes, of which only six were reported to have cognate RDase B genes (Siddaramappa *et al.*, 2012). A similar observation was made for *Dhgm* strain WBC-2 and 20 out of 22 putative RDase A genes do not have accompanying B genes (Molenda *et al.*, 2016). Of the

52 putative RDase genes identified on the genome of strain GP, 10 have an associated B gene. Of the three RDases expressed in VC-grown GP cultures, only CerA is encoded by an RDase A gene with an associated B gene (Supplementary Table 6). The only other expressed RDase A protein encoded by a gene with an adjacent B gene is prokka_01300, a putative 1,1-DCE RDase (Figure 5). The current understanding of organohalide respiration is that RDase B proteins with transmembrane-spanning domains anchor the RDase to the outside of the cytoplasmic membrane (Hug, 2016). The majority (that is, 48 out of 52) of RDase A genes identified on the strain GP genome encode TAT signal peptides indicative of protein transport through the cytoplasmic membrane (Supplementary Table 6). It is currently unclear if RDase A enzymes without accompanying RDase B protein are functional, or if a single RDase B protein can anchor distinct catalytic A units. Expression of RDase B proteins could not be confirmed in proteomic analysis, and it remains to be determined if RDase A genes not associated with an RDase B gene encode RDases functional in organohalide respiration. Interestingly, some RDases (prokka_00862 and prokka_01297) expressed by strain GP are predicted to possess a Sec signal peptide and transmembrane-spanning regions (Supplementary Table 6), suggesting that not all RDase A proteins may require B proteins for catalytic activity. Further investigations are required to derive mechanistic understanding of RDase A interactions with the cytoplasmic membrane.

In contrast to the characterized *Dhgm* isolates, *Dhc* strains cannot utilize formate, although genes encoding subunits of formate dehydrogenase have been annotated in *Dhc* genomes (Key *et al.*, 2017). Due to the lack of the corresponding enzyme activity, the formate dehydrogenase annotation was recently modified to 'complex iron-sulfur molybdoenzyme' (CISM) (Kublik *et al.*, 2016). In *Dhc* strain CBDB1, CISM forms a complex with the RDase and the iron-sulfur cluster containing subunit of the hydrogen uptake hydrogenase Hup. The complex is localized on the outer side of the cytoplasmic membrane and presumably mediates electron transfer from hydrogen to the chlorinated electron acceptor (Kublik *et al.*, 2016). Interestingly, *Dhgm* possesses two different CISM gene clusters (prokka_00355 and prokka_01480) and *Dhgm* may possess two distinct electron transfer complexes. *Dhgm* also possesses the genes encoding heterodisulfide reductase, an electron bifurcation system and *Dhgm* may utilize HdrABC for electron splitting as part of the organohalide respiration process.

The comparative metagenome analysis of consortia ANAS, KB-1 and Donna II, all capable of converting polychlorinated ethenes to ethene, revealed similar functional characteristics based on SEED functional categories, yet little resemblance was observed regarding the relative abundance of non-dechlorinating taxa within each consortium

(Hug *et al.*, 2012). Similar conclusions were derived from the culture GP metagenome analysis, indicating that functional, and not taxonomic, redundancy is the norm in reductively dechlorinating communities. This information may have value to predict the potential success of natural attenuation in environments impacted with chlorinated solvents, where metagenomics can reveal enriched functions. Therefore, additional metagenomes of dechlorinating consortia and environments impacted by chlorinated solvents can further refine the characteristic gene content of microbial communities performing organohalide respiration.

Unique features of dechlorinating culture GP included the persistence of 16S rRNA genes of the bacterial phylum WWE1 during the enrichment process. Phylum WWE1, which was recently proposed as candidate phylum Cloacimonetes (Rinke *et al.*, 2013), was first identified in a municipal anaerobic sludge digester (Chouari *et al.*, 2005). To date, no stable enrichment cultures or isolates representing this phylum have been obtained, likely due to their symbiotic relationships with hydrogenotrophic microorganisms (Chojnacka *et al.*, 2015). Metagenome analysis of a municipal anaerobic sludge digester lead to the assembly of the genome of '*Candidatus* Cloacimonas acidaminovorans', a member of phylum WWE1. Annotation of the genome suggested amino acid fermentation as the organism's main metabolism (Pelletier *et al.*, 2008). In meromictic Sakinaw Lake, the depth-dependent co-occurrence of Chloroflexi, Candidate divisions WWE1, OP9/JS1, OP8 and OD1, and methanogens suggested syntrophic interactions between these groups (Gies *et al.*, 2014). The findings reported in several metagenomic studies support a coexistence pattern between Chloroflexi, candidate phylum WWE1 and methanogens (Wrighton *et al.*, 2012; Hug *et al.*, 2013a; Wrighton *et al.*, 2014). Dechlorinating culture GP harbored *Dhgm*-type Chloroflexi, phylum WWE1 and hydrogenotrophic methanogens, and this community could be maintained in defined, bicarbonate-buffered medium amended with lactate and VC. Thus, culture GP is a potential source for isolating phylum WWE1 representatives.

Collectively, these results demonstrate that pristine (that is, not impacted by anthropogenic chlorinated solvents) habitats (for example, grape pomace compost) harbor organohalide-respiring bacteria. Thus microbiomes associated with pristine ecosystems can be a source of novel RDases such as the VC RDase CerA with potential value for biotechnological applications. The survey of 1173 groundwater samples demonstrated that *Dhgm* commonly occur in contaminated aquifers, and evidence that this bacterial group contributes to VC detoxification has implications for contaminated site assessment, monitoring, and decision making. The findings further emphasize that organohalide-respiring Dehalococcoidia participate in the natural cycling of chlorine and carbon, and highlight that organohalide

respiration occurs in pristine environments. The latter observation has implications for energy-depleted and isolated environments where naturally occurring halogenated hydrocarbons could fuel microbial life.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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