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Development and characterization of DehaloR^2, a novel anaerobic microbial consortium performing rapid dechlorination of TCE to ethene

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Abstract A novel anaerobic consortium, named DehaloR^2, that performs rapid and complete reductive dechlorination of trichloroethene (TCE) to ethene is described. DehaloR^2 was developed from estuarine sediment from the Back River of the Chesapeake Bay and has been stably maintained in the laboratory for over 2 years. Initial sediment microcosms showed incomplete reduction of TCE to DCE with a ratio of *trans*- to *cis*- isomers of 1.67. However, complete reduction to ethene was achieved within 10 days after transfer of the consortium to sediment-free media and was accompanied by a shift to *cis*-DCE as the prevailing intermediate metabolite. The microbial community shifted from dominance of the Proteobacterial phylum in the sediment to *Firmicutes* and *Chloroflexi* in DehaloR^2, containing the genera *Acetobacterium*, *Clostridium*, and the dechlorinators *Dehalococcoides*. Also present were *Spirochaetes*, possible acetogens, and *Geobacter* which encompass previously described dechlorinators. Rates of TCE to ethene reductive dechlorination reached 2.83 mM Cl^- d^{-1} in batch bottles with a *Dehalococcoides* sp. density of 1.54E+11 gene copies per liter, comparing favorably to other enrichment cultures described in the literature and identifying DehaloR^2 as a promising consortium for use in bioremediation of chlorinated ethene-impacted environments.

Keywords *Dehalococcoides* · Chlorinated ethenes · Sediment microorganisms · Reductive dechlorination

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Introduction

Trichloroethene (TCE) is among the most common pollutants at hazardous waste sites (Abelson 1990; McCarty 1997). While it is naturally produced by micro- and macro-organisms in marine environments (Abrahamsson et al. 1995; Kittelmann and Friedrich 2008a), reductively dechlorinating microorganisms from these water sources have not been extensively studied, but could potentially provide a wealth of novel chlorinated ethene-respiring bacteria. Various strains of *Dehalococcoides* sp. are the only identified bacteria to date capable of performing complete dechlorination to ethene (Taş et al. 2009).

Previously reported chlorinated ethene-respiring microbial consortia studied in marine sediment microcosms were distinct from those obtained from soil and groundwater (Griffin et al. 2004; Kittelmann and Friedrich 2008a; Miller et al. 2005). One of the most striking differences was the transformation of perchloroethene (PCE) and TCE to greater amounts of the intermediary *trans*-dicloroethylene (*trans*-DCE), whereas consortia from soil and groundwater microcosms primarily generate *cis*-DCE as an intermediate or end product (Griffin et al. 2004; Kittelmann and Friedrich 2008a; Miller et al. 2005). Furthermore, in most microcosms showing an elevated ratio of *trans*- to *cis*-DCE, both DCE congeners accumulated over time (Griffin et al. 2004; Kittelmann and Friedrich 2008b; Miller et al. 2005), indicating either the absence or inhibition of bacteria able to perform complete dechlorination to ethene.

Various sediment-free, chlorinated ethene-respiring communities have been developed and characterized for application in bioaugmentation (Duhamel and Edwards 2006; Macbeth et al. 2004; Richardson et al. 2002; Schaefer et al. 2009). These cultures have several similar features.

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Most importantly, they all contain at least one strain of *Dehalococcoides*, and most contain an additional member of three genera capable of TCE dechlorination to *cis*-DCE: *Geobacter*, *Dehalobacter*, and *Desulfuromonas*. These previously described microbial consortia are also dominated by bacteria capable of converting fermentable substrates to H_2 and acetate, two metabolites representing, respectively, the required electron donor and carbon source for *Dehalococcoides*. The bacteria responsible for fermentation include at least one species of homoacetogens of the genera *Sporomusa*, *Spirochaetes*, or *Acetobacterium*, believed to provide *Dehalococcoides* with growth factors, including vitamin B_{12} and similar corrinoids that serve as cofactors for their reductive dehalogenase enzymes (Johnson et al. 2009). In addition to the microbial ecology, a significant question for bioremediation of chlorinated solvents is how to compare the kinetics of various chlorinated ethene cultures and how to ascertain the cause of any given differences in chlorinated ethene transformation rates.

In this study, we developed a sediment-free, anaerobic microbial consortium, designated as DehaloR^2, that has been maintained stably in the laboratory for over 2 years and performs reductive dechlorination of TCE to ethene at high rates compared to many similar cultures in the literature. The originating inoculum for this novel consortium was sediment from a brackish tributary of the Chesapeake Bay (CB) near Baltimore, Maryland, where dechlorination products of the antimicrobial compound triclocarban (TCC) previously suggested the presence of dehalorespiring microbiota (Miller et al. 2008). Using pyrosequencing and phylogenetic analyses, we compared the microbial community structure of CB sediment to DehaloR^2. We also characterized DehaloR^2 in terms of various kinetic parameters and investigated the microbial community with a clone library and quantitative PCR (qPCR).

Materials and methods

Development and maintenance of a sediment-free, TCE to ethene dechlorinating culture

The sediment was obtained from the Back River, a tributary of the Chesapeake Bay (CB) located near Baltimore, Maryland. This tidal body of water receives effluent from a wastewater treatment plant. Previous work showed possible evidence of microbial reductive dechlorination activity towards polychlorinated aromatics in sediment from the sampling location utilized for this study (Miller et al. 2008; Heidler et al. 2006). Upon arrival at the laboratory, CB sediment samples from different depths of the core were mixed homogeneously in an anaerobic glove chamber (Coy laboratory products Inc. Grass Lake, MI) in an atmosphere of 3.5% H_2 and 96.5% N_2 and stored in sterile Mason jars at 4°C.

Microcosms were set up in the anaerobic glove chamber by transferring 10 g of sediment into 160 mL glass serum bottles containing 90 mL of sterile anaerobic medium as described by Loffler et al. (2005), 1 mL ATCC vitamin supplement, 50 μg/mL vitamin B_{12}, 2 mM lactate, and 450 μM TCE. Microcosms were sealed with butyl rubber stoppers and incubated statically in the dark at 30°C. Two microcosms were set up with autoclaved sediment as abiotic controls.

To generate sediment-free cultures, we transferred 10 mL liquid suspension to triplicate serum bottles (100 mL total liquid volume) amended with 5 mM lactate, 11.1 mM methanol, and 320 μM TCE. We named this culture DehaloR^2. DehaloR^2 (available through the Arizona State University Arizona Technology Enterprises) has been maintained in our laboratory for over 2 years under the above conditions. When the chlorinated electron acceptors are consumed, the cultures are re-amended with TCE and electron donors and are transferred into fresh media regularly with a 10% inoculum.

Chemical analyses Concentrations of TCE, 1,1-DCE, *cis*-DCE, *trans*-DCE, vinyl chloride (VC), ethene, and methane were quantified by injecting 200 μL headspace samples with 500 μL gas-tight syringes (Hamilton Company, Reno, NV) into a Shimadzu gas chromatograph (GC-2010, Columbia, MD) equipped with a flame ionization detector. We used an Rt™-QSPLOT capillary column (30 m× 0.32 mm×10 μm, Restek, Bellefonte, PA) and helium as the carrier gas. The initial oven temperature was 110°C held for 1 min, and then raised with a gradient of 50°C/min to 200°C. A second gradient at 20°C/min raised the temperature to 200°C, followed by a third gradient at 15°C/min to 220°C and held for 2.5 min. The temperature of the FID and injector were 240°C.

Concentrations of lactate, acetate, propionate, and methanol were measured using a Shimadzu high performance liquid chromatography (HPLC, LC-20AT) equipped with an Aminex HPX-87H (Bio-Rad) column for separation of simple acids and solvents. The eluent was 2.5 mM sulfuric acid fed at a flow rate of 0.6 mL/min. We detected chromatographic peaks using a photodiode-array detector at 210 nm and a refractive index detector. The total elution time was 60 min and the oven temperature was constant at 45°C.

DNA extractions For the pyrosequencing analysis of the sediment, DNA was extracted from 0.4 g of wet sediment. The extraction was carried out with a FastID (Genetic ID NA, Inc., Fairfield, IA) kit, MoBio bead tubes (MoBio Laboratories, Carlsbad, CA), and 10% SDS solution to

increase cell lysis and improve DNA yields. For all other DehaloR^2 DNA extractions, pellets were formed from 1.5 mL liquid centrifuged for 15 min and stored at −20°C. Before proceeding with the DNA extraction protocol for Gram-positive bacteria (QIAGEN DNeasy® Blood and Tissue Kit), we suspended the pellets in 180 μL lysis buffer containing 20 mM Tris–HCl, 2 mM EDTA, 250 μg/mL achromopeptidase, and 20 mg/mL lysozyme, incubated for 60 min at 37°C in a Thermomixer® R (Eppendorf), and treated the suspension with SDS (1.2% *w*/*v*) before a further incubation for 10 min at 56°C. All extracted DNA was quantified with a Nanodrop-1000 instrument (Nano-Drop Techonologies, Inc.).

Genomic-based methods for microbial ecology analysis We amplified genomic DNA with 16S rRNA gene bacteria primers (Zhou et al. 1997) and used the product as a template for nested PCR reactions targeting the 16S rRNA gene of *Dehalobacter* (Schlotelburg et al. 2002) and *Desufuromonas* (Loffler et al. 2000) primers to verify the presence or absence of these organisms. qPCR targeting the 16S rRNA gene was used to quantify bacteria, *Dehalococcoides* and *Geobacteraceae*. Since this region is almost identical across *Dehalococcoides*, we quantified the reductive dehalogenase genes *tceA*, *bvcA*, and *vcrA* to better assess presence of characterized and unique strains (Holmes et al. 2006). We performed TaqMan® triplicate assays and seven-point standard curves in an Eppendorf Realplex 4S realcycler with 4 μL of DNA in 10 μL total reaction volume. The primers, probe, reagent concentrations, and thermocycler conditions for bacteria were described in Ritalahti et al. (2006), in Cummings et al. (2003) for *Geobacteraceae*, and in Holmes et al. (2006) for *Dehalococcoides*. For the reductive dehalogenase genes, we used a triplicate assay described in Lee et al. (2008).

A bacteria clone library was constructed following a protocol outlined by Torres et al. (2009). We trimmed the vector sequences with SeqMan Pro software (DNASTAR, Madison WI) and achieved 73 partial sequences. Out of 73 clones, we selected 9 clones as major phylotypes and completed nearly full-length sequences (>1,480 bp, except for clone DhR^2/LM-G05 at 840 bp) for further analysis. We assessed the quality of the sequences by using chimera check programs Bellerophon (Huber et al. 2004) and Mallard (Ashelford et al. 2006) and compared them to previously published sequences using BLAST search tool. We initially aligned 16S rRNA gene sequences using the Nearest Alignment Space Termination (NAST) program (DeSantis et al. 2006) and improved the alignment by manual correction with the editor ARB_EDIT4 that is based on 16S rRNA gene secondary structure (Ludwig et al. 2004). We constructed a neighbor-joining tree by using the ARB software package (Ludwig et al. 2004) with the Jukes-Cantor corrected distance matrix and the LanemaskPH filter that assists in preventing overestimation of branch lengths (Zhang et al. 2009). Our nine major phylotypes included in the phylogenetic tree were deposited in GenBank under accession numbers HQ012835–HQ012843.

Pyrosequence analyses targeting the V4 region of the 16S rRNA gene were performed on the sediment sample. The combined V2 and V3 regions were the target of duplicate DehaloR^2 samples from the next study, after the fourth reduction of TCE to ethene. For the sediment sample, we used 454 GS-FLX protocols as described by Zhang et al. (2010), and for the DehaloR^2 samples, the bacterial tag-encoded FLX-Titanium amplicon pyrosequencing (bTEFAP) was performed as described by Wolcott et al. (2009). We conducted sequence alignment, clustering, and classification by following procedures explained by Zhang et al. (2010). We used the program "mother" to calculate rarefaction, Shannon Index, and Chao1 estimator (Schloss et al. 2009).

Experiment for determining maximum TCE turnover rates We carried out the experiment in triplicate batch reactors consisting of 160 mL glass serum bottles (100 mL liquid, 60 mL headspace). Initially, 10% DehaloR^2 inoculum from a highly enriched culture with complete conversion to ethene was transferred to each bottle, along with 5 mM lactate and 11.1 mM methanol. We added 10 to 15 μL of neat TCE. The initial pH was between 7.2 and 7.5, and the initial bicarbonate concentration was 30 mM. After complete dechlorination to ethene, we removed 1.5 mL of culture for DNA extraction and 0.5 mL for a protein assay to determine biomass concentration (Bicinchoninic Acid Kit, Sigma-Aldrich, Milwaukee, WI). Before the next addition of lactate, methanol, and TCE, the bottles were flushed with N_2 to remove headspace gases and were amended with 10 mM bicarbonate. TCE and electron donors were added five consecutive times, until the rates of reductive dechlorination, the concentration of biomass, and the cell copies measured with qPCR stabilized and then started to decrease, indicating the onset of biomass decay.

Results

Sediment microcosm and culture development

Figure 1 shows that the sediment microcosms had an 18-day lag time before the onset of dechlorination. TCE was predominantly converted to *trans*-DCE and *cis*-DCE between days 18 and 40, after which reductive dechlorination stalled at a *trans*-to-*cis*-DCE mole ratio of 1.67±0.15, calculated from nine data points over 85 days. The small amount of VC (2.6 μM) and ethene (0.9 μM) indicated only a small

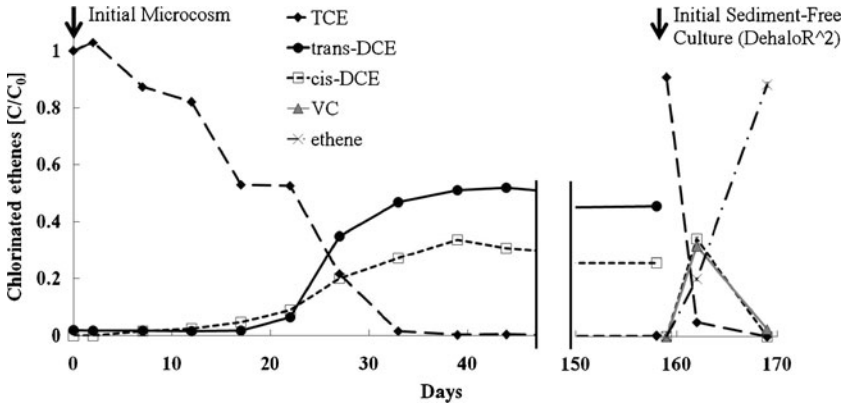

Fig. 1 Chemical conditions in culture vessels showed a shift from incomplete reductive dechlorination of TCE to DCE (*trans*-to-*cis* isomer ratio of 1.67±0.15) in the initial sediment microcosm to complete and much more rapid dechlorination to ethene with negligible accumulation of *trans*-DCE in the first transfer to a sediment-free culture, designated DehaloR^2. Shown are measurements for a representative microcosm and averages of triplicate cultures of DehaloR^2

proportion of DCE was reductively dechlorinated in the sediment. Concomitant measurements of the headspace by GC-FID showed production of a significant amount of methane in the initial microcosms (data not shown). In the abiotic controls, no TCE reductive dechlorination byproducts were detected over the course of the experiment. In contrast to the microcosms, the sediment-free DehaloR^2 consortium rapidly dechlorinated 320 μM TCE to ethene within 10 days with formation of *cis*-DCE as the primary DCE intermediate (from day 160 in Fig. 1).

DehaloR^2 community structure

The structures of the microbial community in the sediment used to set up the microcosms and in duplicate enrichment cultures of DehaloR^2 are shown at phyla and genera levels in Fig. 2. The numbers of trimmed sequence tags were 6,792 for the sediment 3,323 and 3,215 for the enrichment cultures. These were grouped, respectively, into 508, 169, and 144 unique operational taxonomic units (OTUs) based on 95% similarity; this information and statistical analyses are summarized in Table 1. The actual numbers of OTUs, sample richness, estimated by the Chao1 statistical parameter, were 776, 262, and 212. Proteobacteria was the dominant phylum in the sediment sequences, with 72% of all sequences, and it decreased to 0.8–1.7% after enrichment. Firmicutes became the major phylum in DehaloR^2 sequences, at 78–86% in comparison to 1.6% in the sediment sequences. Since most Firmicutes are fermenters, enrichment on excess lactate and methanol (6.7 and 6 me- eq. per TCE addition, respectively) channeled ~80% of the electron equivalents in approximately equal ratios to acetate (~6.5 mM) and propionate (~3.5 mM), thereby biasing the microbial community towards this phylum. The genus *Dehalococcoides* and its corresponding phylum, *Chloroflexi*, increased from non-detectable to 9–16% in DehaloR^2.

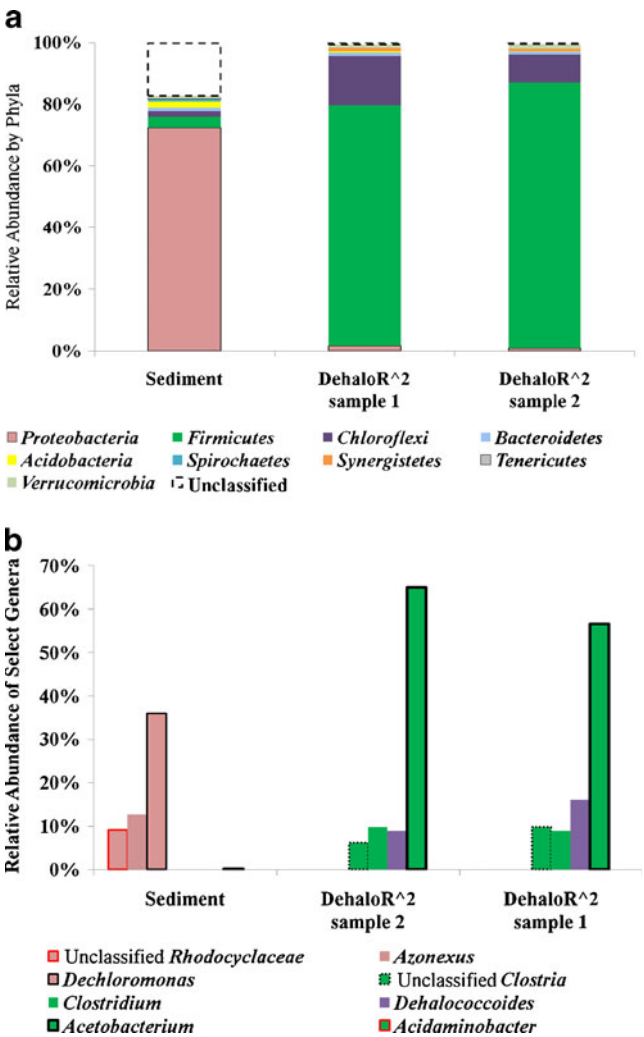

Fig. 2 Comparison of the bacterial communities by (**a**) phyla and (**b**) selected genera in the microcosm sediment and duplicate DehaloR^2 enrichment cultures. Pyrosequencing targeted the V4 region of the 16S rDNA for the sediment and the combined V2 and V3 regions for DehaloR^2

Table 1 Sequence processing data of the microcosm sediment and duplicate DehaloR^2 enrichment cultures

Sample	Trimmed tags	Observed species (OTUs at 0.05 difference)	Chao1 estimator of richness at 0.05 difference (95% CI)	Shannon index H′ estimator of diversity at 0.05 difference (varH)	Evenness at 0.05 difference
Sediment	6792	508	776 (695,891)	4.015 (0.00061)	0.644
DehaloR^2 sample 1	3323	169	262 (220,337)	2.861 (0.00091)	0.558
DehaloR^2 sample 2	3215	144	212 (179,276)	2.581 (0.00107)	0.519

Pyrosequencing targeted the V4 region of the 16S rDNA for the sediment and the combined V2 and V3 regions for DehaloR^2

While the sediment sequences at the genus level were dominated by the Proteobacteria *Dechloromonas* (36%) and *Azonexus* (12.7%), in addition to unclassified Rhodocyclaceae (9.1%), three genera from the Firmicutes phylum were highly enriched in the second transfer of DehaloR^2. *Clostridia* and the homoacetogen *Acetobacterium*, both negligible in the sediment, increased to 8.9–9.9% and 56–65%, respectively. Unclassified Clostridium accounted for 6.2–9.8% of the sequence tags. Additionally, *Geobacter*, a dechlorinating organism capable of transforming TCE to *cis*-DCE, stayed relatively constant at 0.5–1.5%.

In order to identify the bacterial species in DehaloR^2, we constructed the clone library depicted in the phylogenetic tree shown in Fig. 3. Of the 73 sequenced clones, 73% were fermenters, with homoacetogens constituting the largest fraction (48%), of which 31 were *Acetobacterium* and four were *Spirochaetes*. *Clostridium ganghwense*, a bacterium that may ferment lactate to propionate, as suggested by Zhao et al. (2008), was the closest phylogenetic match (97%) to 14 clones (19.2%). Nineteen clones (26.0%) were *Dehalococcoides* sp., which appear to be represented by several strains, some of which may be novel, as suggested by our sequencing data. While the relative abundance of fermenters, including *Acetobacterium*, agreed well with the pyrosequencing analysis, we used qPCR to verify the abundance of *Dehalococcoides* compared to bacteria and found $1.54 \pm 0.27 \times 10^{11}$ and 1.25 0.50×10^{12} gene copies L^{-1}, respectively. We also quantified the cell density of *Geobacteraceae*, $2.67 \pm 0.38 \times 10^{10}$ genes copies L^{-1}, the family containing the

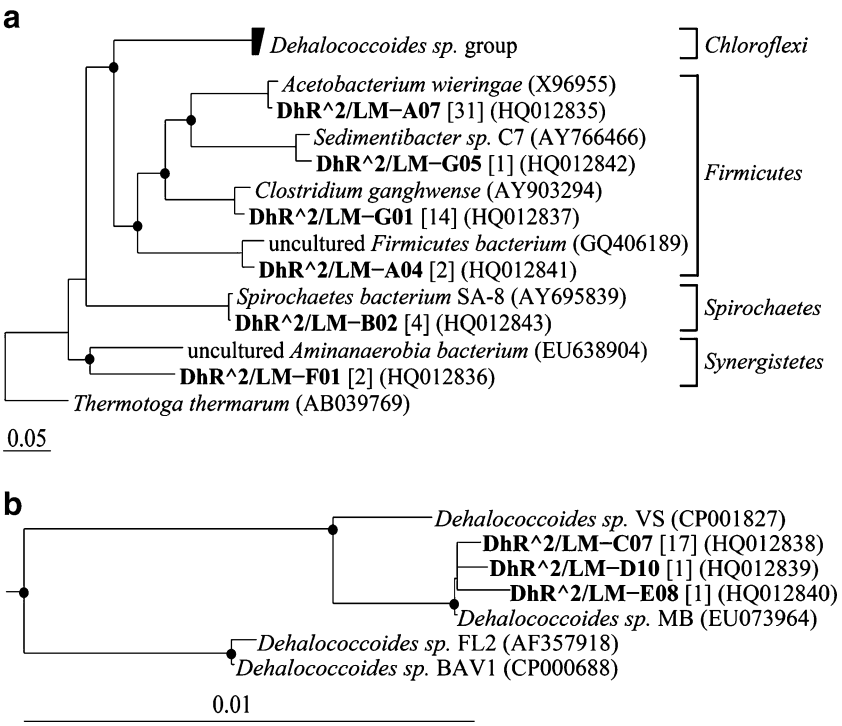

Fig. 3 Neighbor-joining phylogenetic trees of 16S rRNA gene sequences from a clone library of DehaloR^2 with 73 clones. **a** A subset of *Chloroflexi*, *Firmicutes*, *Spirochaetes*, and *Synergistes* phyla based on nearly full-length sequences (>1,480 bp), except for clone DhR^2/LM-G05 (840 bp). *Thermotoga thermarum* was used as the outgroup sequence. **b** An expanded branch illustrating the *Dehalococcoides* sp. group. Bootstraps were calculated based on 1,000 replicates and values greater than 500 are indicated with *filled circles*. Sequences from this study are in *bold*, *brackets* indicate the number of clones that belong to each phylotype, and accession numbers are in *parentheses*. The *scale bars* indicate the number of nucleotide substitutions per site

genus *Geobacter*. *Desulfuromonas* and *Dehalobacter*, two other common TCE to *cis*-DCE dechlorinators, were not detected, even when using specific primers and nested PCR (data not shown), thereby indicating absence of these bacteria. Given our current understanding of reductively dechlorinating anaerobes, the reductive dechlorination activity displayed by DehaloR^2 appears to be linked to *Dehalococcoides* and *Geobacteraceae*. Results from qPCR targeting previously characterized *Dehalococcoides* reductive dehalogenase genes revealed that *vcrA*, *tceA*, and *bvcA* were present in high abundance and were larger than the total amount of *Dehalococcoides*: $1.56 \pm 0.40 \times 10^{11}$, $9.96 \pm 0.41 \times 10^{11}$, and $5.09 \pm 0.78 \times 10^{9}$ gene copies L^{-1}, respectively.

Maximum TCE turnover in DehaloR^2

Comparing the maximum rate of reductive dechlorination can be of practical value when selecting potential cultures for bioaugmentation. The results of an enrichment culture time-course experiment are presented in Fig. 4. The rate of TCE reductive dechlorination to ethene ($\Delta C\ \Delta t^{-1}$) was calculated for each addition of TCE, and the maximum rate, 2.83 mM Cl^- d^{-1}, was reached in the third addition when the concentration of *Dehalococcoides* had also reached a maximum (Fig. 4). This rate is compared to other values in the literature in Table 2.

Discussion

This work produced a novel microbial consortium, DehaloR^2, for potential use in bioaugmentation of chlorinated ethene spill sites. Its enrichment history is of interest for the following three reasons. First, the origin was estuarine sediment. Second, whereas the chlorinated ethene-degrading microcosm from which DehaloR^2 was developed stalled at DCE with significant production of *trans*-DCE, complete dechlorination through *cis*-DCE was attained when sediment was precluded from the culture (Fig. 1). This is the first report of achieving complete dechlorination to ethene after transferring an incompletely dechlorinating sediment consortium to a sediment-free culture medium. The immediate onset and rapid dechlorination to ethene suggests that *Dehalococcoides* sp. capable of complete dechlorination to ethene were present in the microcosm but were inhibited by sediment constituents. The cause for this inhibition and the accumulation of *trans*-DCE is presently unknown and continues to be the subject of investigation. Third, while many reductively dechlorinating cultures were developed from PCE and TCE spill sites (Duhamel and Edwards 2006; Macbeth et al. 2004; Richardson et al. 2002; Schaefer et al. 2009), DehaloR^2 originated from sediment that, to our best knowledge, had not before been exposed to chlorinated ethene contaminants (Miller et al. 2008). The sediment utilized in this study has not yet been characterized extensively for its chemical composition, and only the presence of two antimicrobial agents, triclosan and triclocarban, have been reported, along with several dechlorination products of triclocarban.

We achieved a deep understanding of the community structure using an emerging, high-throughput sequencing technique, pyrosequencing, a powerful tool for microbial ecologists. To our knowledge, pyrosequencing has only been used to characterize one other TCE dechlorinating microbial community (Zhang et al. 2010). While in Zhang et al. (2010) the target was the V6 region of the 16S rRNA gene which only allowed differentiation of the bacteria at the genus level, here we targeted the combined V2 and V3

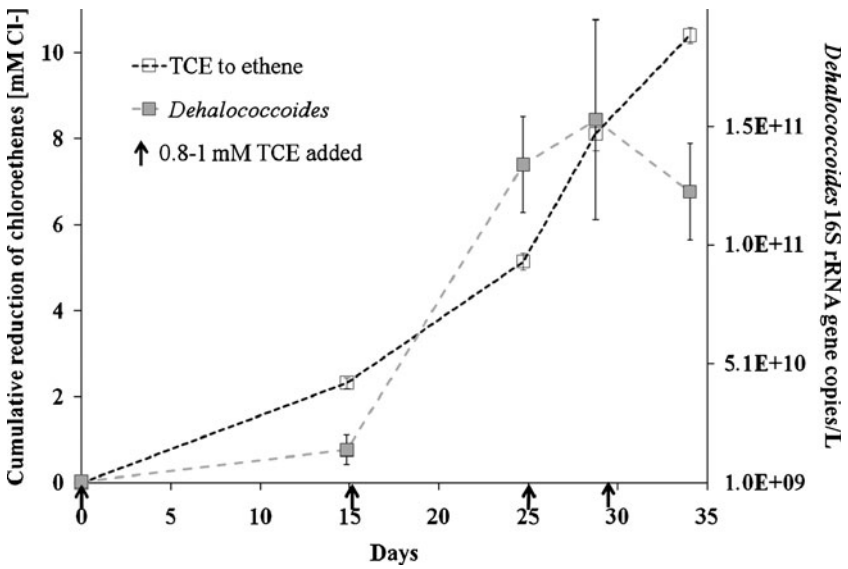

Fig. 4 Kinetic experiment of successive TCE additions in triplicate enrichment cultures plotted in terms of the cumulative reduction of TCE to ethene, as millimolars Cl^- produced, and the concentration of *Dehalococcoides*. Note that 1 mM Cl^- is released during each step in the reductive dechlorination

Table 2 Comparison of maximum chlorinated ethene turnover rates $(\Delta C\ \Delta t^{-1})_{max}$ to ethene and the corresponding concentration of *Dehalococcoides* (X_{Dhc}), for selected chlorinated ethene mixed microbial communities in batch serum bottles

Culture	$(\Delta C\ \Delta t^{-1})_{max}$ [mM Cl^- d^{-1}]	X_{Dhc} [cells L^{-1}]	$(\Delta C\ \Delta t^{-1})_{max}\ X_{Dhc}^{-1}$ [mmol Cl^- $cell^{-1}$ d^{-1}]
DehaloR^2	2.83±0.60 (TCE)	$1.54 \pm 0.27 \times 10^{11}$	$1.9 \pm 0.11 \times 10^{-11}$
SDC-9[a]	2.9 (PCE),	1.4×10^{11}	2.1×10^{-11}
Unnamed[b]	0.96 (PCE)	–	–
VS[c]	0.31 (VC)	4.0×10^{11}	7.8×10^{-13}
KB1[d]	0.16 (TCE)	8×10^{10}	–
ANAS	0.006[e] (TCE), 0.05[f] (TCE)	$1.0 \pm 0.29 \times 10^{10}$[e]	6×10^{-13}
BDI[g]	0.03 (TCE)	1×10^{11}	–

The turnover rate per *Dehalococcoides* cell was only calculated when values for $(\Delta C\ \Delta t^{-1})_{max}$ and X_{Dhc} were available from the same source and where data were from the stationary phase

[a] Vainberg et al. (2009), $(\Delta C\ \Delta t^{-1})_{max}$ was calculated from Fig. 4 and X_{Dhc} from Table 1

[b] Xiu et al. (2010)

[c] Cupples et al. (2004)

[d] Haest et al. (2010), $(\Delta C\ \Delta t^{-1})_{max}$ and X_{Dhc} were calculated from Fig. 2, and X_{Dhc} was the final concentration of cells

[e] Freeborn et al. (2005), $(\Delta C\ \Delta t^{-1})_{max}$ was from Fig. 1 and X_{Dhc} from Table 3

[f] Richardson et al. (2002), calculated assuming 200 μmol TCE/bottle were reduced in 10 days

[g] Amos et al. (2008), $(\Delta C\ \Delta t^{-1})_{max}$ was calculated from Fig. 2 and X_{Dhc} was the final concentration of cells in Fig. 1

regions, allowing us to identify organisms at the species level. We found that the pyrosequencing results correlated well with the microbial community in the clone library, providing over 3,000 sequences compared to 73 in the clone library.

The relative abundance of *Dehalococcoides* sp. in DehaloR^2, determined at 26% in the clone library and 9–16% in the pyrosequencing of the enrichment cultures, was not surprising compared to other mixed cultures in the literature grown on similar concentrations of chlorinated ethenes (Duhamel and Edwards 2006; Duhamel and Edwards 2007; Richardson et al. 2002). However, it was surprising that the sum of *vcrA*, *tceA*, and *bvcA* obtained with qPCR was larger than the concentration of *Dehalococcoides*, as all described *Dehalococcoides* strains have only one of these reductive dehalogenase genes. qPCR data and the 16S rRNA gene clone library suggest the presence of novel *Dehalococcoides* strains. Laboratory efforts are underway to further characterize these potentially novel organisms all of which, based on sequence homology of the clone library and pyrosequencing, belong to the *Dehalococcoides* Cornell group.

Another interesting quality of the community structure was the relative abundance of specific homoacetogen genera and species when compared to previous research. Homoacetogens may affect rates of reductive dechlorination by providing cofactors to the *Dehalococcoides* reductive dehalogenase enzymes (Johnson et al. 2009). *Acetobacterium* was dominant in the pyroseqeuncing enrichment cultures (56–65%), and in the clone library, they were 42.4%, with *Spirochaetes* contributing 5.5%. In an in situ remediation field study by Macbeth et al. (2004), in which a TCE plume was amended with lactate as the electron donor, *Acetobacterium* comprised 34.4% of the bacterial clone library community and *Spirochaetes* contributed 4.3%. Richardson et al. (2002) identified a relative abundance of 20% Eubacterium (the class containing *Acetobacterium*) and 1.1% *Spirochaetes* in a TCE-transforming mixed culture, ANAS, which utilizes lactate as the electron donor. In the TCE-transforming mixed culture KB1, *Acetobacterium* was not dominant when the consortium was maintained with methanol as the electron donor (Duhamel and Edwards 2006; Duhamel and Edwards 2007). Duhamel and Edwards (2006) initially reported a relative abundance of *Spirochaetes* at 10.8% as the only homoacetogen in KB1 and later reported a composition of 26% *Sporomusa*, 12% Spirochaetes (taxonomic class), and 1% *Acetobacterium* (Duhamel and Edwards 2007). The comparable relative abundance of homoacetogens between mixed cultures is a function of providing mostly identical medium with similar concentrations of fermentable substrates, while the specific genera and species are reflective of the culture sediment, soil, or groundwater origin.

A key finding is that the maximum turnover rate of TCE to ethene $(\Delta C\ \Delta t^{-1})_{max}$ in DehaloR^2 was at least one order of magnitude larger than other cultures reported in the literature, except for culture SDC-9 (Table 2) (Amos et al. 2008; Cupples et al. 2004; Haest et al. 2010; Richardson et al. 2002; Vainberg et al. 2009; Xiu et al. 2010). The value of $(\Delta C\ \Delta t^{-1})_{max}$ is reached in the stationary phase when X_{Dhc} is at its maximum for a given culture, and so the maximum turnover rate per cell $(\Delta C\ \Delta t^{-1})_{max}\ X_{Dhc}^{-1}$ was calculated when data were available. X_{Dhc} for

DehaloR^2 was the same order of magnitude as those of other cultures and pure strains (Table 2), but only SDC-9 matched the high value of $(\Delta C\ \Delta t^{-1})_{max}\ X_{Dhc}{}^{-1}$ in DehaloR^2, suggesting that the fast metabolic rates in DehaloR^2 are a function of enzymatic kinetics and not of its ability to reach higher densities of dechlorinators.

In summary, we characterize here the first dechlorinating culture from estuarine sediment able to reduce TCE to the harmless compound ethene, with rapid rates of reductive dechlorination that are not attributable to the concentration of *Dehalococcoides*. It will be worthwhile to investigate whether this is due to the presence of novel dechlorinators, novel reductive dehalogenase enzymes, optimal cofactors provided to the reductive dehalogenase enzymes from other members in the community, or a combination of the above.

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