

Supporting Information

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SI Materials and Methods

Cultivation and Isolation. Completely defined, anaerobic mineral salts medium for bacterial enrichment and isolation was prepared as described (1–3). Enrichment of polychlorinated biphenyl (PCB)-dechlorinating *Dehalococcoides* was achieved by sequential transfers in 100 mL medium in 160-mL serum bottles supplemented with sodium lactate (10 mM) and the chlorinated substrate, 30 ppm (80.65 μ M) Aroclor 1260 or 0.7 mM tetrachloroethene (PCE). Further isolation of *Dehalococcoides* strains was conducted by serial dilutions in 20-mL vials filled with 10 mL of medium amended with 10 mM sodium acetate as the carbon source, hydrogen as the electron donor (5×10^4 Pa), and 0.7 mM PCE as the electron acceptor. The dilution-to-extinction experiments used 50 ppm ampicillin to control growth of nonresistant bacteria. In pure cultures, 5 ppm single PCB congeners (i.e., 234-234-CB, 234-245-CB, 236-245-CB, 245-245-CB, 2345-234-CB, 2345-236-CB, 2345-245-CB, and 2356-245-CB) were added to verify PCB dechlorination pathways. Unless stated otherwise, cultures were incubated at 30 °C in the dark without shaking.

Analytical Techniques. PCBs were extracted with isoctane and quantified by gas chromatography equipped with an electron capture detector (Agilent) and a DB-5 capillary column (J&W Scientific) as described (1, 2). Chlorinated ethenes were analyzed by gas chromatography using flame ionization detector and a GS-GasPro column (2).

DNA/RNA Extraction, PCR Amplification, Clone Library Construction, and qPCR. Cells were harvested by centrifugation (15 min, $10,000 \times g$, 4 °C). DNA and RNA for qPCR and genomic analyses were extracted from 1 mL and 800 mL cultures, respectively, using QIAGEN DNeasy Blood and Tissue Kit and RNeasy mini kit (QIAGEN). Repeated attempts to extract RNA from PCB-fed pure cultures failed to generate enough RNA for RNA-seq libraries. Therefore, PCB-fed mixed cultures were used for RNA extraction for metatranscriptomic analyses of PCB dechlorination. RNA for qPCR analyses was reverse transcribed into cDNA (cDNA) using a two-step RT-PCR Sensiscript kit as described (4). PCR amplifications with RDase gene-specific primers (Dataset S3) were conducted on a Mastercycler cycler (Eppendorf) under conditions as described previously (4). Four clone libraries of 16S rRNA, adenosine kinase (*adk*), ATP synthase delta chain (*atpD*), and beta subunit of bacterial RNA polymerase (*rpoB*) genes were constructed using the TOPO-TA cloning kit (Invitrogen). qPCR (Applied Biosystems) enumeration of *Dehalococcoides* cells and RDase genes was performed with QuantiTect SYBR Green PCR kit (4).

Sequencing and Genome Assembly. Illumina sequencing of the 16S rRNA genes was conducted for the PCB- and PCE-dechlorinating mixed cultures as described previously (1).

Genomic DNA was extracted from PCE-fed pure cultures and fragmented using Adaptive Focused Acoustics (Covaris). DNA sequencing libraries were then prepared and multiplexed using TruSeq DNA Sample Prep kit (V2) prior 2×76 paired end sequencing. Bases with quality scores lower than 3 were trimmed off the 3' ends of reads and read pairs with a read shorter than 60 bp were discarded. Contig assembly was performed with SOAPdenovo (5) and scaffolding with Opera (6). Reads were down-sampled to around 100 \times coverage because this was seen to improve assembly statistics (N50). We tried different k-mer sizes and the assembly with the least number of scaffolds, and highest

N50 was kept. GapCloser (5) was used for in silico closing of gaps between contigs. Finally, targeted PCR reactions and Sanger sequencing were used to confirm the orientation and position of scaffolds and close remaining gaps to obtain a closed circular genome. Metagenomic sequencing of mixed cultures was also performed and assembled.

RNA integrity for transcriptomic and metatranscriptomic analyses was assessed using Bioanalyzer (Agilent). Encore Complete Prokaryotic RNA-Seq DR Multiplex System kit (Nugen) incorporating a selective rRNA cleavage procedure was used for library building. Sequencing reads were also preprocessed through trimming and filtering as described for genome sequencing. The processed reads were mapped to their respective reference genomes, and PCR duplicates were removed using Picard's (<http://picard.sourceforge.net>) MarkDuplicates function before quantification as reads per kbp per million (RPKM).

ORF Prediction and Annotation. ORFs were predicted using Prodigal (7). Functional annotations were assigned by screening predicted ORFs with entries in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (8) using RapSearch (9). Genes for rRNAs and tRNAs were identified using RNAmmer (10) and tRNAscan-SE (11). The three novel isolate genomes were further annotated using rapid annotation using subsystem technology (RAST) (12). Predicted ORFs for the metagenomics libraries were annotated with average read coverage (estimated from mapped reads) and analyzed with metagenomics RAST's assembled pipeline (13).

Polymorphism Analysis from Metagenomic Data. For metagenomic SNP-based analyses, reads from the metagenomes were first mapped to the desired reference genome. Then, LoFreq (14) was used to call genome-wide SNPs with default parameters and assign frequencies to each SNP. MetaPhlAn (15) was used to analyze the metagenomic reads for relative abundance of *Dehalococcoides* in mixed cultures.

Identification of RDases. For the identification of *rdh* genes in closed genomes, 306 *rdhA* genes were downloaded from the National Center for Biotechnology Information (NCBI) protein database, aligned with Clustal-Omega (16), and converted into a hidden Markov model (HMM) that was used to search against the predicted ORFs with HMMER3 (<http://hmmer.org>). Finally, the predicted *rdhA* genes were blasted against the NCBI non-redundant (NR) database and manually confirmed. *RdhB* genes were identified in the same way. Potential *rdhA* fragments in the metagenomic assembly were identified using nhmmer and the consensus *rdhA* HMM model described above. Identified fragments larger than 250 bp (default inclusion threshold defined by nhmmer) were considered for analysis. Final confirmation of the *rdhA* fragments was performed via manual curation from basic local alignment search tool results against the NCBI NR database. Reads from metagenomes of PCB-/PCE-fed mixed cultures were then mapped to the respective fragments, and mapped counts were normalized to RPKM values.

Phylogenetic Analysis. Phylogenetic analysis was performed by aligning genomes against the reference genome (strain 195) using nucleotide mummer (17) to call SNPs (alignments shorter than 10 kbp were ignored). The corresponding reference-based multiple alignment of genomes (restricted to variant positions) was analyzed using PhyML (18) to construct maximum likelihood trees with 100 bootstrap replicates.

RdhA phylogenetic analysis was performed with 192 *rdhA* protein sequences derived from the five previously published *Dehalococcoides* strains and strains CG1, CG4, and CG5. Multiple sequence alignment of the *rdhA* protein sequences was performed using Clustal Omega (16). Bayesian inference of the *rdhA* phylogeny was done with MrBayes (19) with mixed amino acid rate matrix (aamodelpr), single chain per analysis (nchains = 1), and 300,000 generations (ngen = 300,000).

Enzymatic Analyses. Cells were harvested from 500 mL cultures by centrifugation (15 min, 10,000 × g, 4 °C) and resuspended in buffer with 10 mM Tris-HCl and 1 mM DTT (pH 7.2). Crude cell lysates were obtained by disruption for 3 min at 40% amplitude with 5 s/10 s working/cooling pulse using a VCX 130 µL trasonicator (Sonics and Materials Inc.). In vitro activity test was performed under anaerobic conditions in 20-mL serum bottles. Each bottle contained 4 mL of assay solution (buffered with 100 mM Tris-HCl, pH 7.0) consisting of 20 mM of methyl viologen that was reduced by 15 mM Ti(III)-citrate, 0.2 mL of cell extract, and the chlorinated compound (15 ppm Aroclor 1260, 15 ppm 2345-245-CB, or 40 µM PCE). Assay bottles were then incubated at 30 °C in the dark before PCB/PCE analyses as described above. Native polyacrylamide gel electrophoresis (PAGE) was used to resolve proteins from crude cell lysates of PCE-fed pure cultures as described (4). Briefly, the native PAGE gels with a 10% resolving gel and a 4% stacking gel were run at 80–160 V

for 2 h at 4 °C under aerobic conditions in an SDS-free buffer (25 mM Tris, 250 mM Glycine, and pH 8.3). The positions of the respective excised protein bands were determined as described (20). The in-gel activity assays were performed in the similar bottles with the in vitro activity tests amended with 2345-245-CB/PCE. The protein bands identified to show both PCB and PCE dechlorination activities were sent to the Protein and Proteomics Centre, Singapore, for tryptic digestion and protein sequencing as described previously (4).

Data Deposition. Raw sequencing reads were deposited into NCBI's short read archive (SRA). Reads for whole-genome sequencing of strains CG1, CG4, and CG5 are accessible using SRA IDs SRR1050472, SRR1050378, and SRR1050473, respectively. The corresponding transcriptomic dataset can be found using IDs SRR1055187, SRR1055188, and SRR1055189.

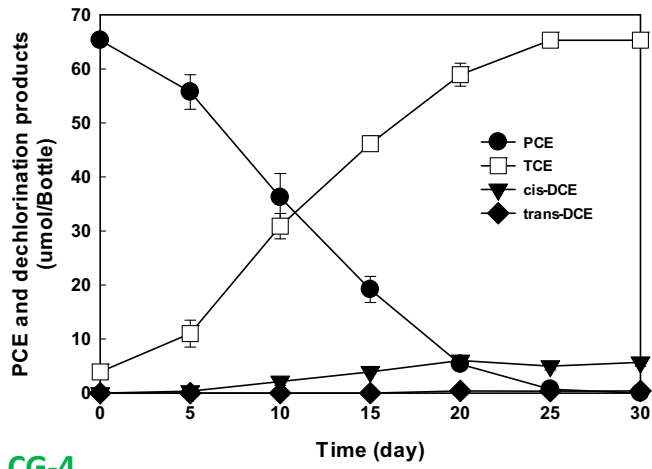
Metagenomics samples for PCB/PCE-fed cultures CG-1, CG-4, and CG-5 were assigned SRA IDs SRR1050524/SRR1050525, SRR1050519/SRR1050523, and SRR1050527/SRR1050528, respectively. Metatranscriptomics for samples CG-1, CG-4, and CG-5 can be downloaded using IDs SRR1051208, SRR1051209, and SRR1051210.

Closed genomes were uploaded to GenBank and assigned IDs CP006949, CP006950, and CP006951 for strains CG1, CG4, and CG5, respectively.

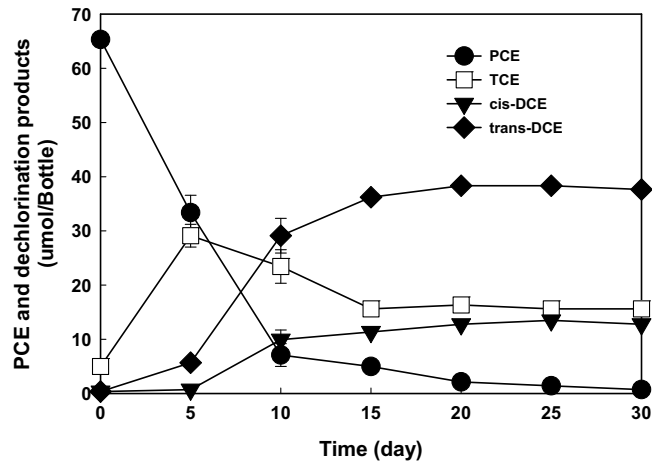
1. Wang S, He J (2013) Phylogenetically distinct bacteria involve extensive dechlorination of aroclor 1260 in sediment-free cultures. *PLoS ONE* 8(3):e59178.
2. Wang S, He J (2013) Dechlorination of commercial PCBs and other multiple halogenated compounds by a sediment-free culture containing *Dehalococcoides* and *Dehalobacter*. *Environ Sci Technol* 47(18):10526–10534.
3. He J, Ritalahti KM, Yang KL, Koenigsberg SS, Löffler FE (2003) Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium. *Nature* 424(6944):62–65.
4. Chow WL, Cheng D, Wang S, He J (2010) Identification and transcriptional analysis of *trans*-DCE-producing reductive dehalogenases in *Dehalococcoides* species. *ISME J* 4(8):1020–1030.
5. Li R, et al. (2010) De novo assembly of human genomes with massively parallel short read sequencing. *Genome Res* 20(2):265–272.
6. Gao S, Sung WK, Nagarajan N (2011) Opera: Reconstructing optimal genomic scaffolds with high-throughput paired-end sequences. *J Comput Biol* 18(11):1681–1691.
7. Hyatt D, et al. (2010) Prodigal: Prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119.
8. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M (2012) KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res* 40(Database issue):D109–D114.
9. Zhao Y, Tang H, Ye Y (2012) RAPSearch2: A fast and memory-efficient protein similarity search tool for next-generation sequencing data. *Bioinformatics* 28(1):125–126.
10. Lagesen K, et al. (2007) RNAmmer: Consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 35(9):3100–3108.
11. Lowe TM, Eddy SR (1997) tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 25(5):955–964.
12. Aziz RK, et al. (2008) The RAST Server: Rapid annotations using subsystems technology. *BMC Genomics* 9:75.
13. Meyer F, et al. (2008) The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* 9:386.
14. Wilm A, et al. (2012) LoFreq: A sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. *Nucleic Acids Res* 40(22):11189–11201.
15. Segata N, et al. (2012) Metagenomic microbial community profiling using unique clade-specific marker genes. *Nat Methods* 9(8):811–814.
16. Sievers F, et al. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7:539.
17. Kurtz S, et al. (2004) Versatile and open software for comparing large genomes. *Genome Biol* 5(2):R12.
18. Guindon S, et al. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst Biol* 59(3):307–321.
19. Ronquist F, et al. (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61(3):539–542.
20. Adrian L, Rahnenführer J, Gobom J, Hölscher T (2007) Identification of a chlorobenzene reductive dehalogenase in *Dehalococcoides* sp. strain CBDB1. *Appl Environ Microbiol* 73(23):7717–7724.

A

CG-1



CG-4



CG-5

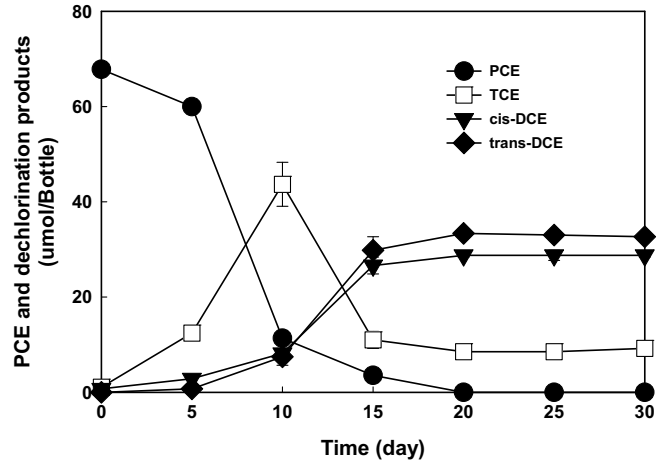


Fig. S1. (Continued)

B

Relative abundances (%) of predominant bacterial genera*						
CG1-PCB	CG4-PCB	CG5-PCB	CG1-PCE	CG4-PCE	CG5-PCE	
11.12	3.37	14.80	85.29	44.29	86.38	<i>Chloroflexi, Dehalococcoidetes, Dehalococcoidales, Dehalococcoidaceae, Dehalococcoides</i>
0.01	0.05	13.39	0.00	0.00	1.94	<i>Synergistetes, Synergistia, Synergistales, Aminiphilaceae, Aminiphilus</i>
9.29	0.51	0.24	0.84	0.06	1.65	<i>Synergistetes, Synergistia, Synergistales, Synergistaceae, TTA_B6, unclassified TTA_B6</i>
0.18	0.09	1.39	0.06	0.02	1.39	<i>Proteobacteria, Deltaproteobacteria, Syntrophobacteriales, Desulfobacteraceae, unclassified Desulfobacteraceae</i>
0.84	0.02	3.97	0.07	0.00	1.25	<i>Spirochaetes, Spirochaetes, Spirochaetales, Spirochaetaceae, unclassified Spirochaetaceae</i>
5.39	2.22	1.13	0.52	0.97	0.74	<i>Firmicutes, Clostridia, Clostridiales, Clostridiales Family XI, Incertae Sedis, unclassified Incertae Sedis</i>
2.88	0.14	1.98	0.84	0.06	0.69	<i>Chloroflexi, Anaerolineae, Anaerolineales, Anaerolineaceae, Levillea</i>
9.89	2.73	2.03	1.89	1.58	0.69	<i>Thermotogae, Thermotogae, Thermotogales, Thermotogaceae, Kosmotoga</i>
9.10	7.82	4.52	1.51	1.93	0.54	<i>Chloroflexi, Anaerolineae, Anaerolineales, Anaerolineaceae, unclassified Anaerolineaceae</i>
0.00	0.03	1.69	0.01	0.01	0.47	<i>Firmicutes, Clostridia, Clostridiales, Clostridiales Family XI, Incertae Sedis, Dethiosulfatibacter</i>
0.73	0.04	5.58	0.18	0.00	0.31	<i>unclassified_Bacteria</i>
2.66	0.04	4.38	0.05	0.00	0.27	<i>Firmicutes, Clostridia, Clostridiales, Clostridiaceae, Clostridium</i>
2.32	0.20	2.42	0.16	0.69	0.24	<i>Proteobacteria, Deltaproteobacteria, Desulfobacteriales, Desulfobacteriaceae, unclassified Desulfobacteriaceae</i>
0.68	19.01	1.27	0.46	21.80	0.14	<i>Firmicutes, Clostridia, Clostridiales, Eubacteriaceae, unclassified Eubacteriaceae</i>
7.59	4.12	0.60	0.70	0.51	0.10	<i>Bacteroidetes, Bacteroidia, Bacteroidales, Porphyromonadaceae, unclassified Porphyromonadaceae</i>
1.05	1.41	2.30	0.05	0.01	0.02	<i>Proteobacteria, Deltaproteobacteria, Syntrophorhabdaceae, unclassified Syntrophorhabdaceae</i>
3.42	4.18	15.11	1.59	2.69	0.01	<i>Synergistetes, Synergistia, Synergistales, Dethiosulfobacteriaceae, unclassified Dethiosulfobacteriaceae</i>
0.17	1.69	0.06	0.02	0.71	0.01	<i>Synergistetes, Synergistia, Synergistales, Thermovirgaceae, Synergistes</i>
0.00	7.75	0.04	0.02	0.16	0.01	<i>Firmicutes, Clostridia, Clostridiales, Clostridiales Family XI, Incertae Sedis, unclassified Incertae Sedis</i>
4.76	0.04	0.21	0.15	0.01	0.01	<i>Synergistetes, Synergistia, Synergistales, Synergistaceae, TTA_B6, unclassified TTA_B6</i>
1.97	0.51	1.61	0.02	0.02	0.01	<i>Proteobacteria, Deltaproteobacteria, Syntrophorhabdaceae, Syntrophorhabdus</i>
8.09	0.60	0.00	0.34	0.01	0.01	<i>Proteobacteria, Deltaproteobacteria, Syntrophobacteriales, Desulfobacteraceae, unclassified Desulfobacteraceae</i>
0.00	0.02	8.36	0.02	0.00	0.01	<i>unclassified_Bacteria</i>
2.30	0.00	1.14	0.01	0.00	0.01	<i>Proteobacteria, Deltaproteobacteria, Syntrophobacteriales, Syntrophaceae, Syntrophus</i>
0.00	8.43	0.00	0.00	16.40	0.00	<i>Firmicutes, Clostridia, Clostridiales, Eubacteriaceae, unclassified Eubacteriaceae</i>
2.14	0.15	0.49	0.01	0.00	0.00	<i>Proteobacteria, Deltaproteobacteria, Syntrophobacteriales, Syntrophaceae, Smithella</i>
1.01	23.88	0.01	0.01	4.09	0.00	<i>Spirochaetes, WWE1, PBS-18, SHA-4, unclassified SHA-4</i>
0.00	6.76	0.00	0.00	1.90	0.00	<i>Thermotogae, Thermotogae, Thermotogales, Thermotogaceae, Fervidobacterium</i>

Fig. S1. (Continued)

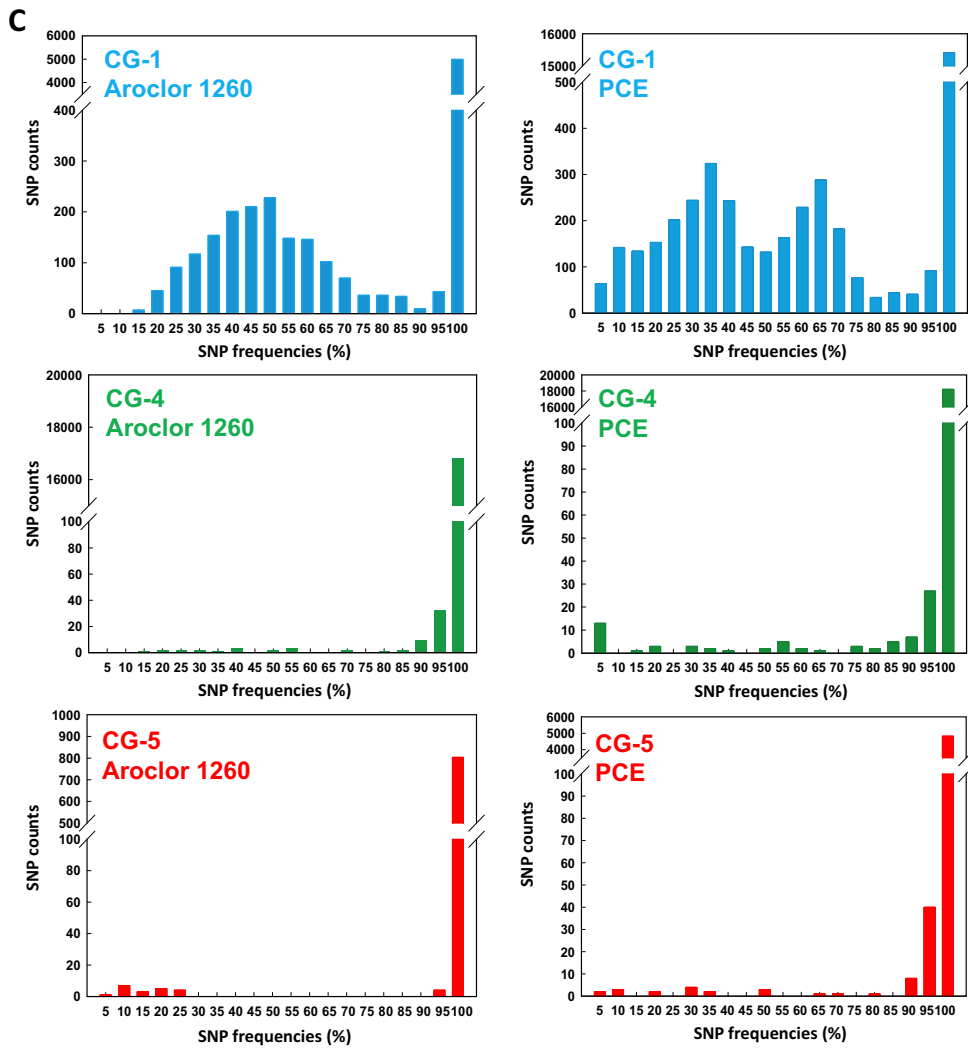


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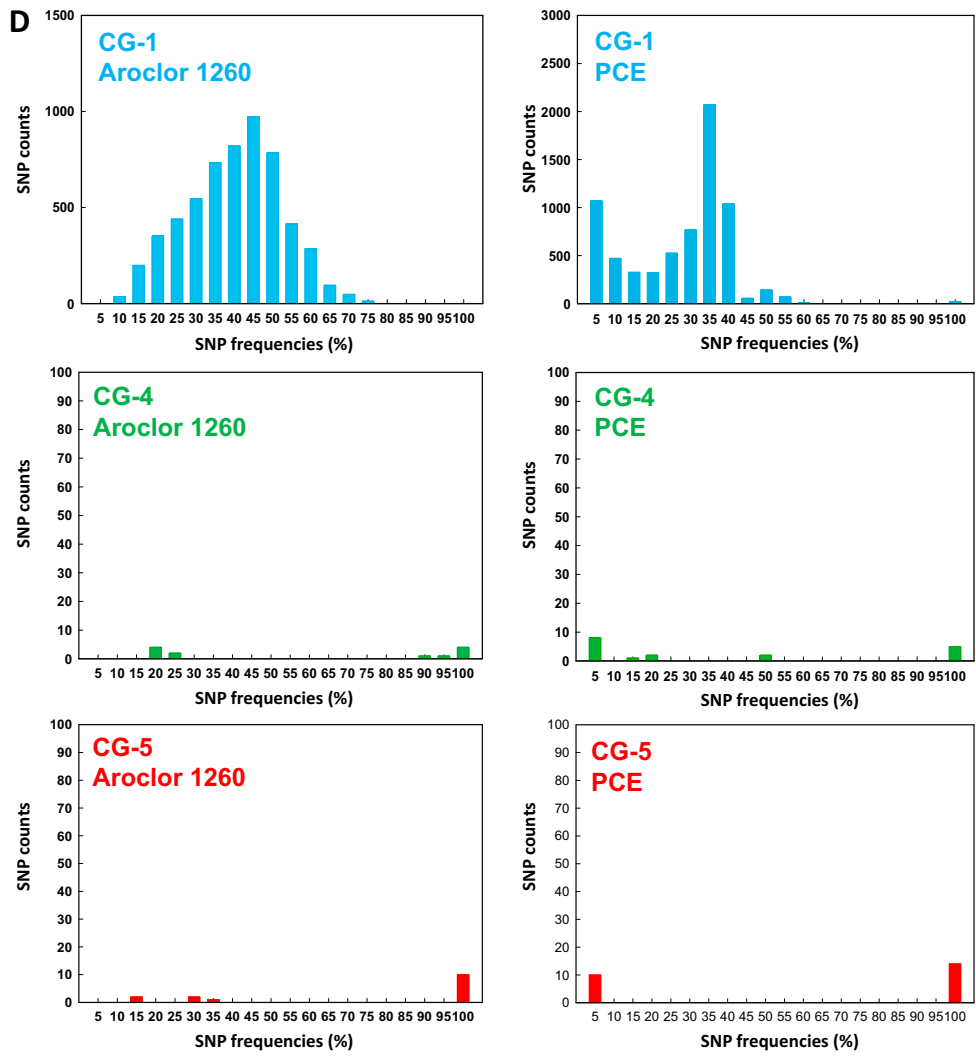


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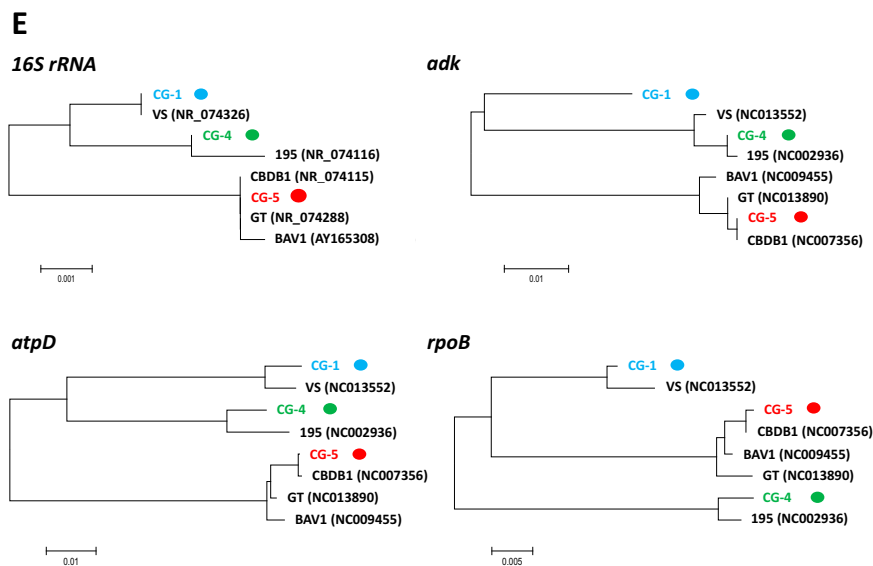


Fig. S1. Kinetic and metagenomic analyses establish the suitability of using PCE as an alternative electron acceptor to enrich and isolate PCB-dechlorinating *Dehalococcoides*. (A) PCE dechlorination to TCE and DCEs in mixed cultures CG-1, CG-4, and CG-5. Error bars represent SDs of triplicate cultures. (B) Genus-level microbial community compositions in PCB- and PCE-fed cultures. *Dehalococcoides* (highlighted in red) becomes the dominant population in all cultures after a single transfer on PCE. Only genera with relative abundances higher than 1.0% in one or more of the cultures are shown. (C) Metagenomic SNP profile for PCB- and PCE-fed mixed cultures amended with lactate as carbon source. SNPs were called against the reference strain 195. The sole peaks at 100% frequency in PCB-/PCE-fed cultures CG-4 and CG-5 suggest that each of the two cultures likely contains a single *Dehalococcoides* strain. The PCB-/PCE-fed culture CG-1 clearly shows at least one more strain with ~50% SNP frequency. The segregation of SNP frequencies into two peaks (other than the one at 100%) in PCE-fed culture CG-1 likely indicates the preferential growth on PCE of one *Dehalococcoides* strain over the other. (D) Metagenomic SNP profiles for PCB- and PCE-fed mixed cultures called against the genomes of *Dehalococcoides* strains isolated from the mixed cultures. The low counts at all SNP frequencies in cultures CG-4 and CG-5 suggest that the isolated *Dehalococcoides* are the only strains in these mixed cultures. In culture CG1, the *D. mccartyi* strain corresponding to the ~65% SNP frequency peak appears to have been enriched and isolated, whereas the other strain in the community retains its SNPs. (E) Neighbor-joining tree of 16S rRNA, *adk*, *atpD*, and *rpoB* gene sequences after global alignment. The scale bar represents changes per nucleotide position. The sequences obtained in this study are marked with solid circles (strain CG1 in light blue, strain CG4 in green, and strain CG5 in red). Culture purities of the *Dehalococcoides* strains were verified by obtaining single genotype sequences from clone libraries targeting the four housekeeping genes.

A

Culture	CG-1					CG-4					CG-5				
	<i>ortho</i>		<i>meta</i>		<i>para</i>	<i>ortho</i>		<i>meta</i>		<i>para</i>	<i>ortho</i>		<i>meta</i>		<i>para</i>
Chlorine position	22'	66'	33'	55'	44'	22'	66'	33'	55'	44'	22'	66'	33'	55'	44'
Abiotic control	1.97	0.56	1.15	1.38	1.32	1.97	0.56	1.15	1.38	1.32	1.97	0.56	1.15	1.38	1.32
Active culture	1.95	0.56	0.96	1.34	1.28	1.94	0.56	1.10	1.30	1.13	1.94	0.54	0.71	0.92	1.24
Change	-0.02	0.00	-0.19	-0.04	-0.04	-0.03	0.00	-0.05	-0.08	-0.19	-0.03	-0.02	-0.44	-0.46	-0.08

B

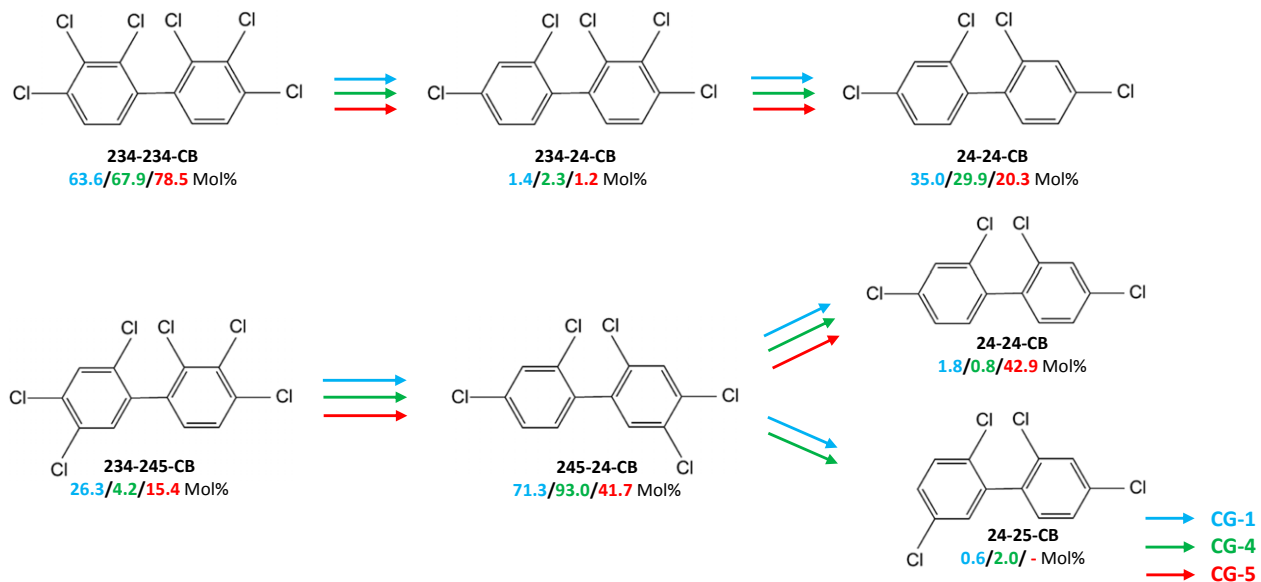
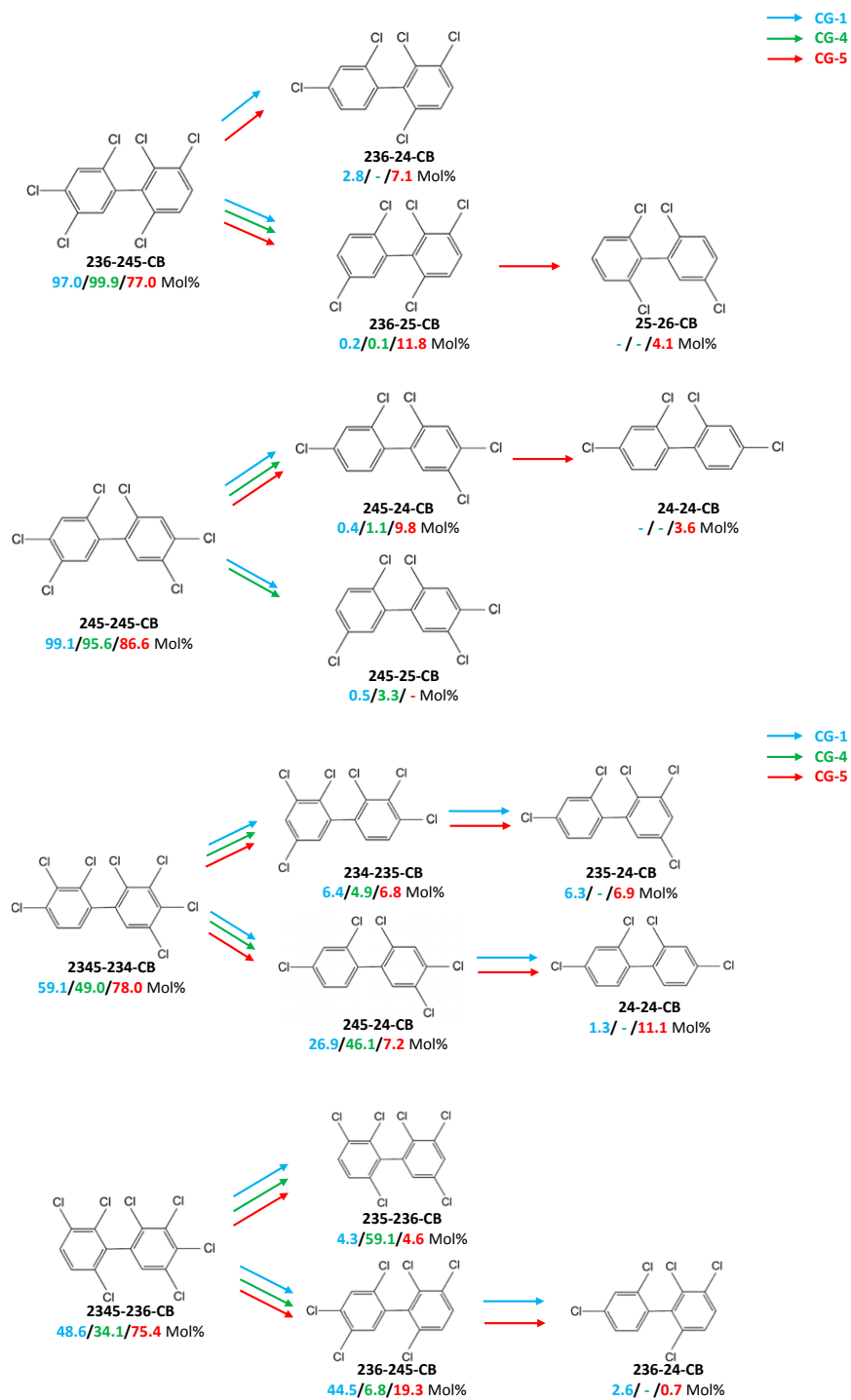


Fig. S2. (Continued)



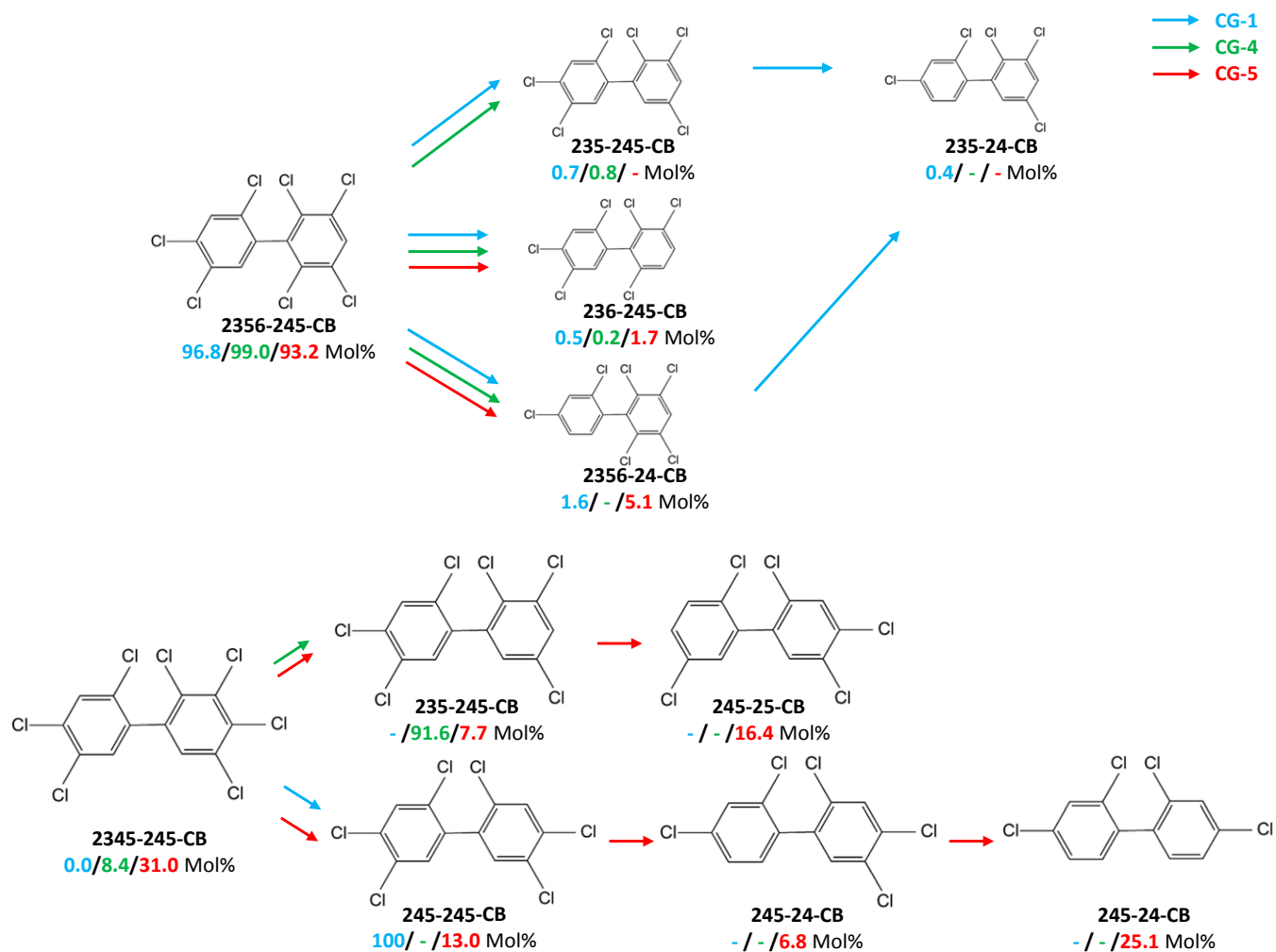


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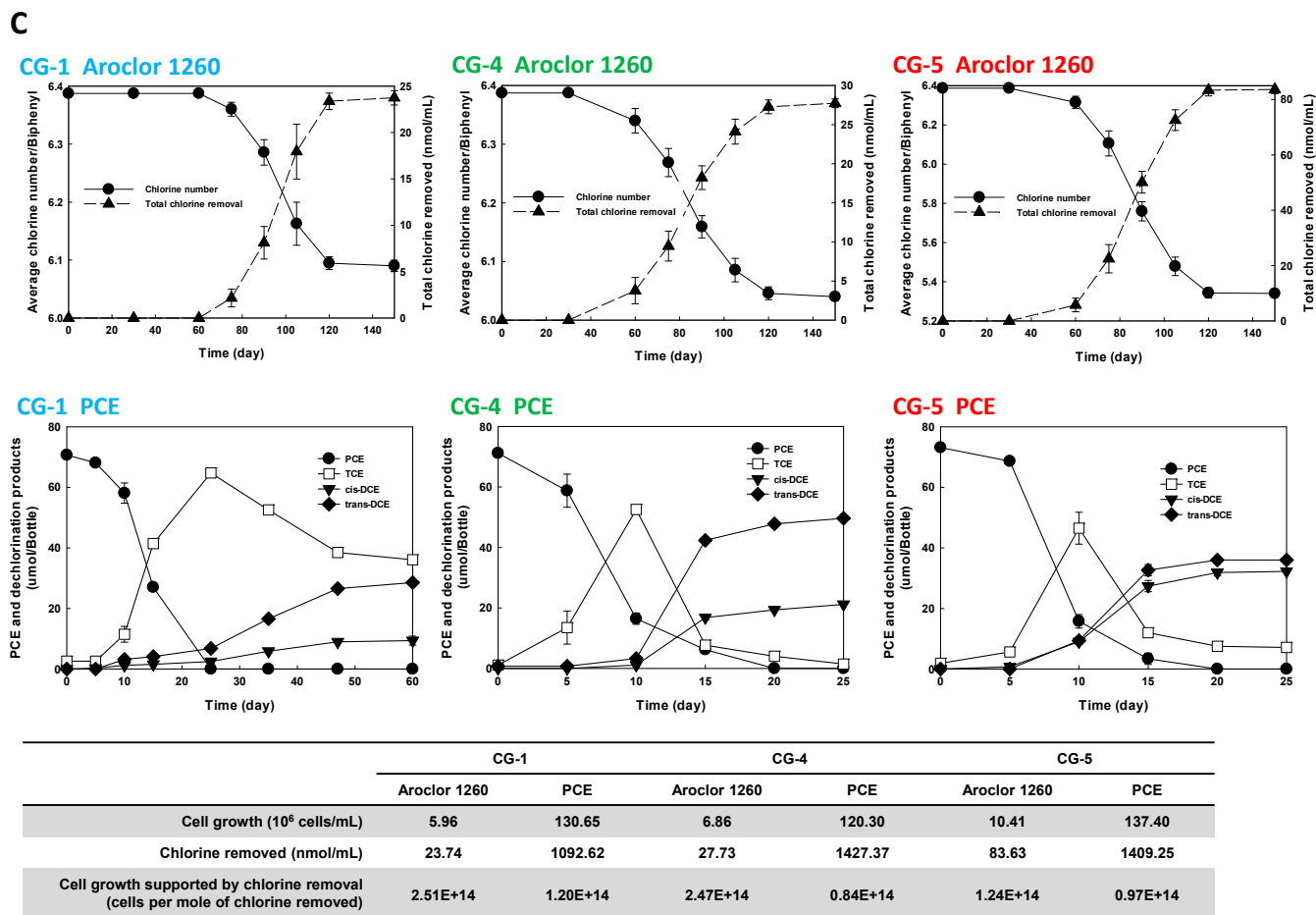


Fig. S2. Distinct dechlorination pathways (A and B) and PCB- and PCE-dependent growth (C) of the three *Dehalococcoides* isolates. (A) Chlorine removal from *ortho*-, *meta*-, and *para*-positions of PCB congeners of Aroclor 1260 and (B) dechlorination pathways of eight PCB single congeners in pure cultures after 6 mo of incubation. The pathways in cultures CG-1, CG-4, and CG-5 are marked in light blue, green, and red, respectively. (C) The growth-coupled chlorine removal from PCB- and PCE-fed pure cultures: (Top) Total chlorine removal and change of average number of chlorines per biphenyl of Aroclor 1260 PCB congeners. (Middle) Profiles of PCE dechlorination to TCE and DCEs. Error bars represent SDs of triplicate cultures. (Bottom) Calculation of cell growth supported by chlorine removal from PCBs and PCE.

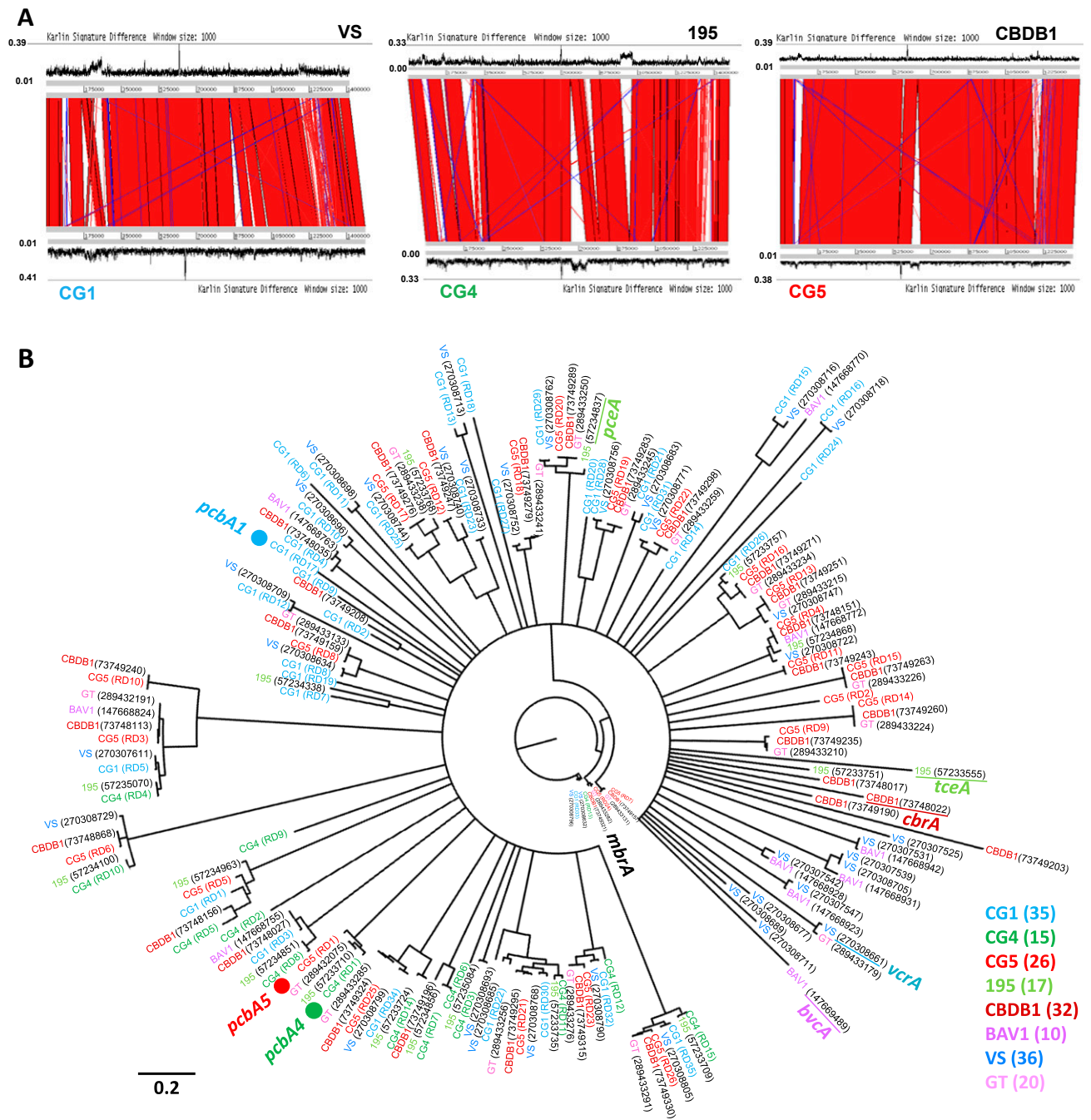


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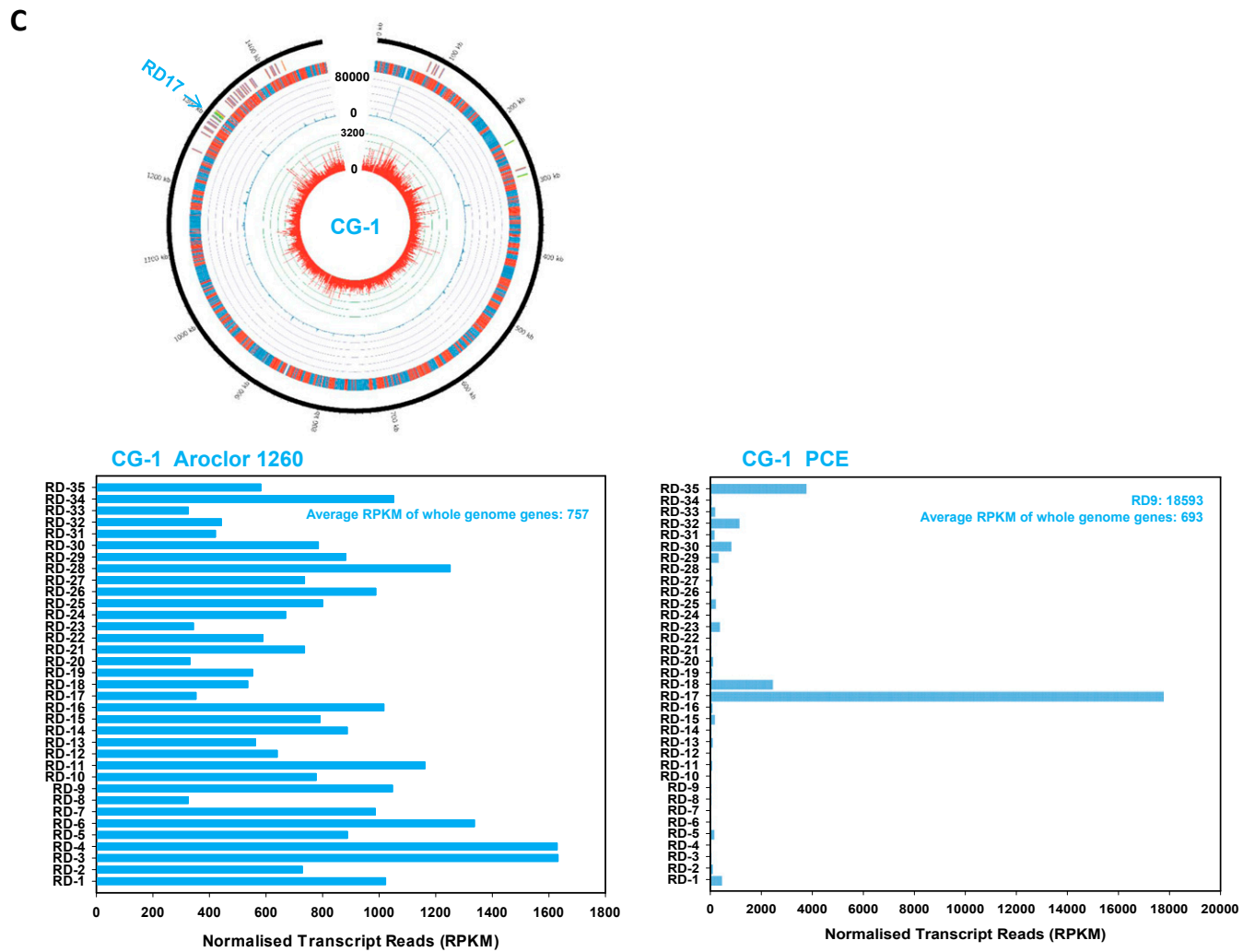
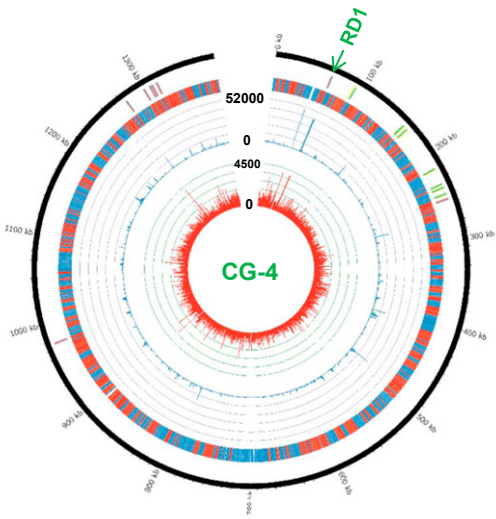
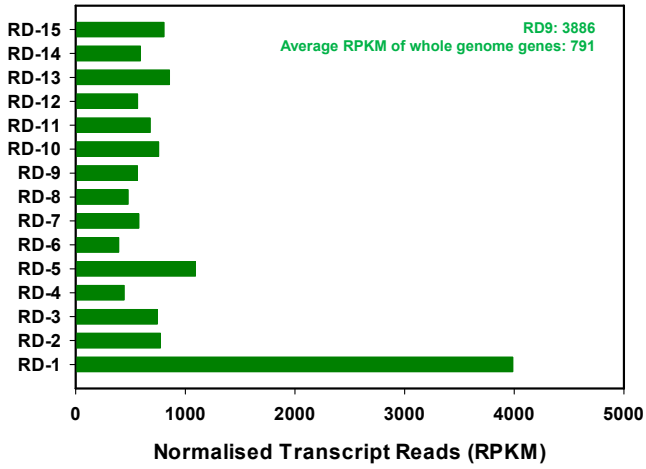


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CG-4 Aroclor 1260



CG-4 PCE

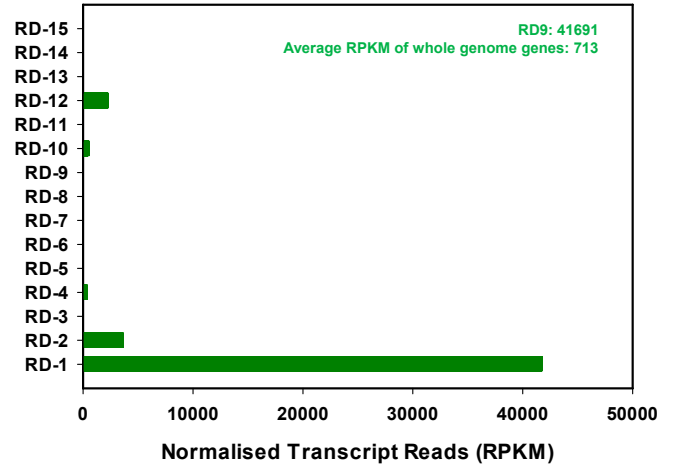


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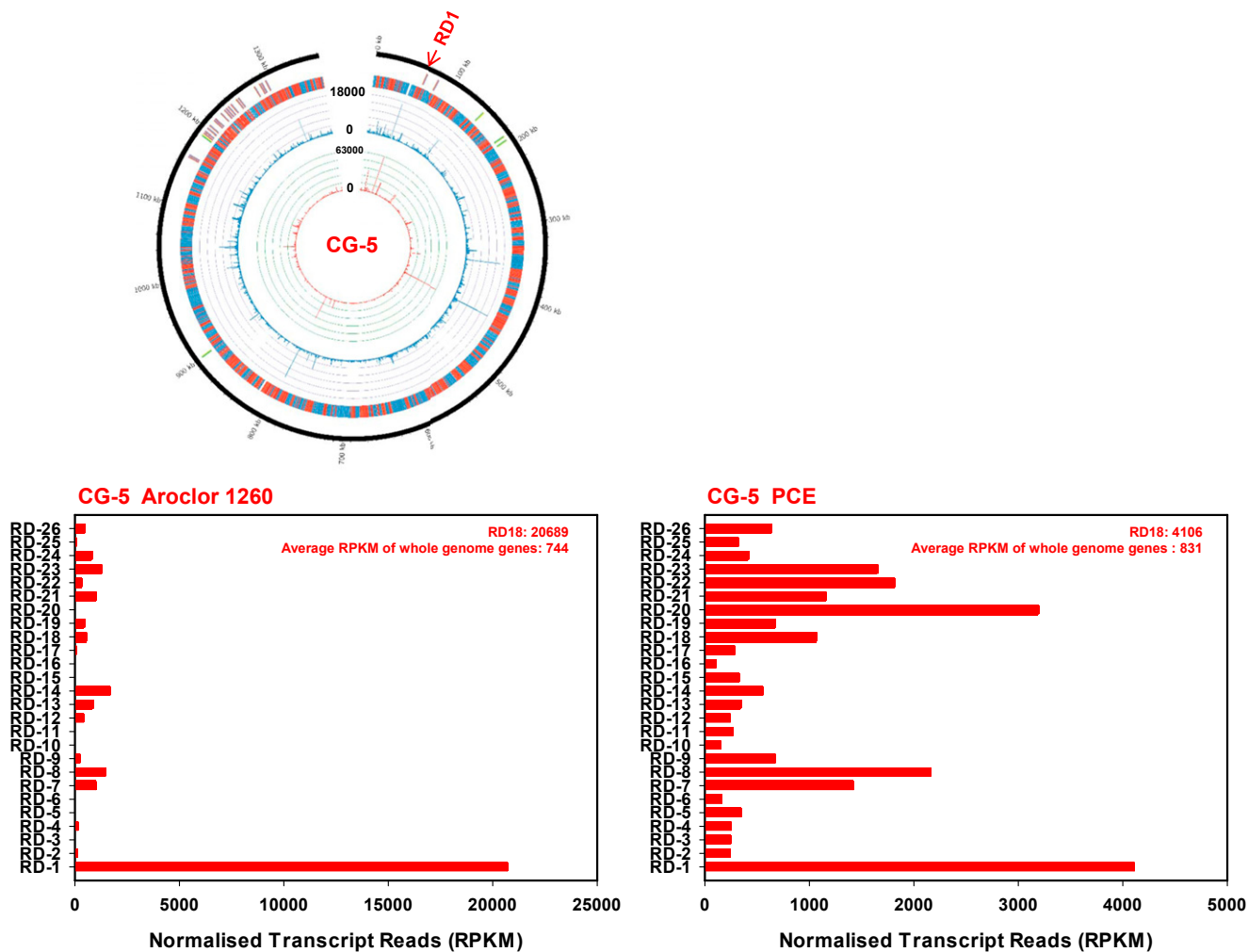
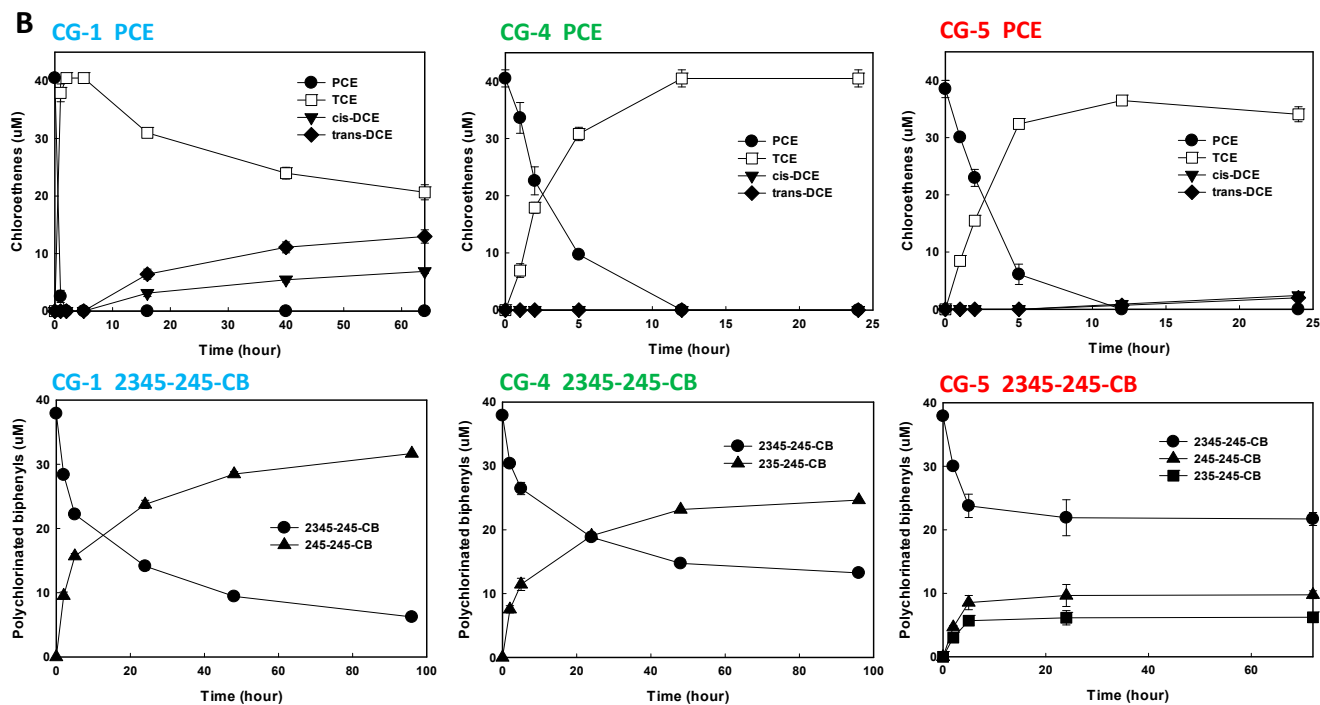
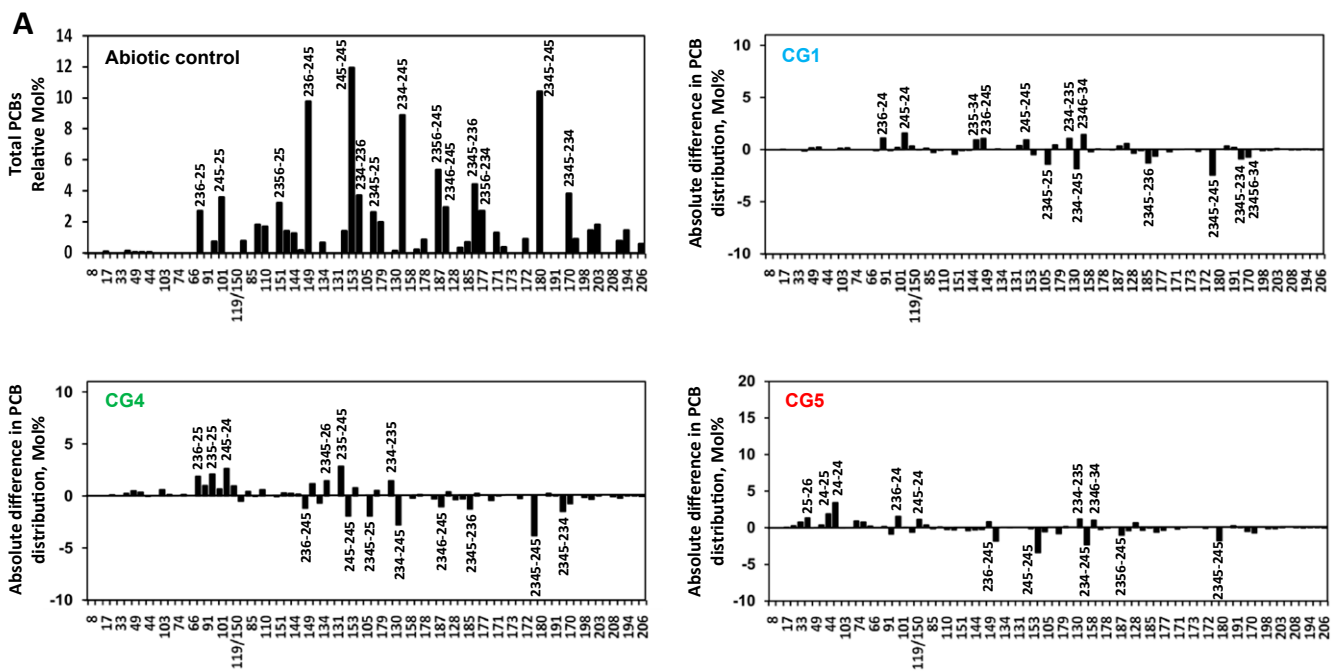


Fig. S3. Genomic and transcriptional analyses of the three *Dehalococcoides* strains. (A) Regions of significant sequence identity between the genomes of strains CG1, CG4, and CG5 and the respective representative *Dehalococcoides* strain in the same subgroups depicted by red and blue (reverse complement) lines. (B) Phylogeny of *rdhA* gene sequences from the eight *D. mccartyi* strains. Deduced amino acid sequences of all full-length *rdhA* genes from the eight strains were analyzed here, and *rdhA* proteins identified to catalyze chlorine removal from both PCBs and PCE are marked with solid circles. (C) Circos plot showing the transcription levels of all genes and bar graphs depicting *rdhA* gene-specific transcription levels for culture CG-1, CG-4, and CG-5. Tracks from inside to outside: track 1 in red, *Dehalococcoides* in PCB-fed mixed culture; track 2 in blue, *Dehalococcoides* in PCE-fed mixed pure culture; track 3, predicted ORFs in the *Dehalococcoides* genome (colors: blue, forward strand; red, reverse strand); and track 4, predicted *rdhABs* (colors: green, *rdhA* on forward strand; purple, *rdhA* on reverse strand; yellow, *rdhB* on forward strand; orange, *rdhB* on reverse strand). Reads are normalized to account for fragment size and library sequencing depth (RPKM).



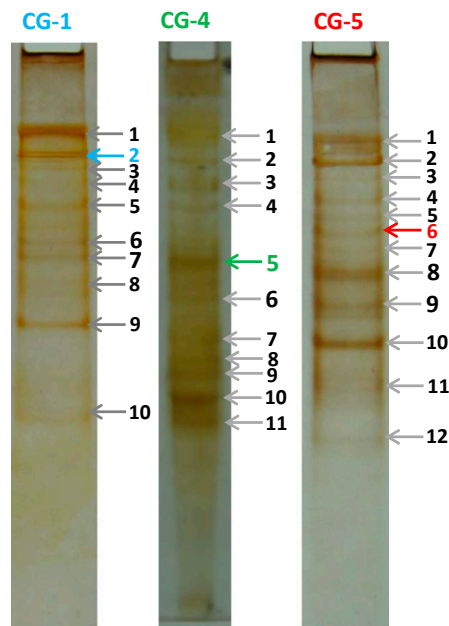
	CG-1		CG-4		CG-5	
	2345-245-CB	PCE	2345-245-CB	PCE	2345-245-CB	PCE
Protein concentration ($\mu\text{g/mL}$)	103.7	103.7	93.3	93.3	74.0	74.0
Chlorine removed (nmol/mL) **	9.6	40.5	7.5	17.9	7.9	15.5
Dechlorination rate ($\mu\text{mol g}^{-1} \text{h}^{-1}$)	46.3	195.3	40.1	95.9	53.4	103.4

* The concentration of 2345-245-CB in the in vitro assay bottles were 37.9 μM (15 ppm).

** The chlorine removed and dechlorination rates were calculated based on first two hours for PCE and 2345-245-CB dechlorination.

Fig. S4. (Continued)

C



Band No.	CG-1			CG-4			CG-5		
	2345-245-CB	PCE	<i>rdhA</i>	2345-245-CB	PCE	<i>rdhA</i>	2345-245-CB	PCE	<i>rdhA</i>
1	-	-	-	-	-	-	-	-	-
2	37.8 μM 2345-245-CB, 0.04 μM 245-245-CB	39.6 μM PCE, 0.5 μM TCE	<i>pcbA1</i>	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
5	-	-	-	37.1 μM 2345-245-CB, 0.8 μM 235-245-CB	35.2 μM PCE, 4.8 μM TCE	<i>pcbA4</i>	-	39.3 μM PCE, 0.6 μM TCE	-
6	-	-	-	-	38.2 μM PCE, 1.7 μM TCE	-	37.6 μM 2345-245-CB, 0.15 μM 245-245-CB, 0.05 μM 235-245-CB	38.9 μM PCE, 1.0 μM TCE	<i>pcbA5</i>
7	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-
11	n.a.	n.a.	-	-	-	-	-	-	-
12	n.a.	n.a.	-	n.a.	n.a.	-	-	-	-

* The dechlorination activities were tested after incubating the gel bands for 24 hours.

** "-" indicates no detectable dechlorination activity; "n.a.", not applicable.

*** The *rdhAs* with PCB- and PCE-dechlorination activities were confirmed through MALDI-TOF/TOF-MS analysis.

Fig. S4. Confirmation of PCB- and PCE-RDases through enzymatic assays. (A) Crude cell lysates of PCE-fed pure cultures catalyze chlorine removal from Aroclor 1260 in the same dechlorination patterns as their active PCB dechlorinating cultures. Absolute difference in the congener distribution of Aroclor 1260 residues between the control without cell lysates and cell lysates of cultures CG1, CG4, and CG5 after 48 h of incubation. Negative mol % indicates the amount of PCBs being dechlorinated and positive numbers represent produced PCB congeners. (B) Dechlorination of PCE and 2345-245-CB in crude cell lysates and calculation of PCB and PCE dechlorination rate by the crude cell lysates. Error bars represent SDs of triplicate assays. (C) Separation of whole cell proteins from PCE-fed pure cultures by native PAGE and in-gel activity test on PCE and 2345-245-CB. The marked protein bands were excised from native PAGE gels and subjected to subsequent in-gel activity assays. The protein samples showing PCB and PCE dechlorination activities were sent for protein sequencing to verify the PCB RDase genes.

Dataset S1. Metagenomic analyses of PCB- and PCE-dechlorinating cultures

Dataset S1

(A) List of KEGG annotated enzymes present in metagenomic libraries of PCB- and PCE-fed mixed cultures CG-1, CG-4, and CG-5. The list is ordered according to their relative abundances (highest to lowest). (B) MetaPhlan microbial community profiling of PCB- and PCE-fed mixed cultures using metagenomic sequencing data. Only microbial genera that are classified as significantly abundant (>0.5% in all three PCB-fed cultures or >0.2% in all three PCE-fed cultures) are shown here. (C) List of novel genes in genomes of CG1, CG4, and CG5 compared with their respective closest *Dehalococcoides* strains (VS, 195, and CBDB1).

Dataset S2. Normalized mRNA transcription values (RPKM) for all predicted ORFs in cultures CG-1, CG-4, and CG-5

Dataset S2

Data are derived from metatranscriptomics of PCB-fed mixed cultures and transcriptomics of PCE-fed pure cultures.

Dataset S3. Real-time PCR primers specifically targeting each *rdhA* gene of strains CG1, CG4, and CG5

Dataset S3

The identified *pcbA1*, *pcbA4*, and *pcbA5* gene-specific primers are shaded with light blue, green, and red, respectively.