Pöritz et al., *Dehalococcoides mccartyi* strain DCMB5 Respires a Broad Spectrum of Chlorinated Aromatic Compounds

Supplemental Material

This supplemental material includes the protocols of the proteome analyses, details of the chemical analyses, the supplemental Table S2 and the supplementary figures S1-S5.

Proteome analyses. Identification of tryptic peptides from cultures grown with PCE was carried out by nano-high-pressure liquid chromatography-coupled mass spectrometry using a Dionex Ultimate 3000 nanoflow uHPLC system (Thermo Scientific, San Jose, CA, USA) coupled to an Orbitrap Fusion mass spectrometer (Thermo Scientific). The sample was loaded on a trapping cartridge (µ-precolumn, 300 µm inner diameter, 5 µm C18). Peptide separation was carried out on a C18 reversed-phase column (Acclaim PepMap RSLC, 15 cm, 2 µm particle size) at a constant flow-rate of 300 nl/min (solvent A: 0.1% formic acid) and linear increasing concentrations of solvent B (80% acetonitrile, 0.08% formic acid) from 4 to 55% during 90 min. Eluting peptides were injected by electrospray ionization into the Orbitrap Fusion mass spectrometer. The following settings were applied for detection of peptide masses in data-dependent mode: precursor masses were determined in the Orbitrap mass analyzer within a mass range of 400-1,700 *m/z* at 60,000 resolution with an automatic gain control (AGC) target of $4x10^5$ and maximum fill time of 50 ms. Precursor masses for MS/MS were selected based on highest intensity (top speed, 3 sec cycle time) and excluded from further MS/MS for 30 s to prevent redundancy in scans. After higher energy collisional (HCD) fragmentation, fragment masses were scanned in the Orbitrap mass analyzer starting from first mass 100 *m/z* at a resolution of 15,000 with $5x10^4$ AGC target and a maximum injection time of 120 ms.

Tryptic peptides from PeCB and TCB cultured samples were analyzed using a nano-flow uHPLC system (nanoLC Ultra model 845, Eksigent, The Netherlands) coupled to a LTQ Orbitrap XL (Thermo Scientific). Peptides were loaded on a trapping column (nanoAcquity UPLC, Waters, 2 cm, 5 µm C18), and eluted from the analytical column (nanoAcquity UPLC, Waters, C18 1.7 µm, 75 µm × 25 cm) with a constant flow-rate of 0.3 µl (0.1% formic acid) using a gradient of 4 to 40% solvent B (0.1% formic acid in acetonitrile) over 150 min. Eluting peptides were ionized by electrospray and analyzed by a full scan in the Orbitrap mass analyzer with 60,000 resolution inside a mass range from 300 to 1,600 *m/z*. The top 6 intense

masses were selected for MS/MS analysis. After collision-induced dissociation (CID), fragment masses were detected in the linear ion trap.

LC-MS data were analyzed using Proteome Discoverer (v1.4.1.14, Thermo Scientific). Two search engines, MS Amanda and Sequest HT, were used to match spectra against the proteome of *D. mccartyi* DCMB5 (containing 1,477 sequences entries, downloaded March 2014 from NCBI) with the following settings: trypsin as cleavage enzyme, oxidation of methionine (Met) as dynamic and carbamidomethylation on cysteine residues (Cys) as static modification, up to two missed cleavages, precursor mass tolerance set to 10 ppm and fragment mass tolerance to 0.5 Da for linear ion trap or 0.05 Da for Orbitrap MS/MS spectra, respectively.

Details of the GC analyses of chlorinated compounds.

Chlorinated benzenes were quantified using a Shimadzu gas chromatograph 2010 with a flame ionization detector (GC-FID) and a Rtx®35 capillary column (30 m length, 0.32 mm i.d., 0.5 µm film thickness; Restek, Bad Homburg, Germany). 1,3,5-Tribromobenzene was used as internal standard (2.5 mM, final concentration 500 µM). Samples were injected with a split of 1:10. The temperature program was modified from (2): initial hold at 40°C for 1.1 min, increase to 70°C at a rate of 40°C per min (1.9 min), increase to 140°C at a rate of 20°C per min (1.2 min), 40°C per min to 160°C (1.2 min), increase to 220°C at a rate of 25°C per min (0.5 min), 40°C per min to 280°C (final hold 5 min). Injector and detector temperatures were 250°C and 280°C, respectively.

Monochlorinated and non-chlorinated dibenzo-*p*-dioxin were analyzed from the headspace of the cultures by solid phase microextraction (SPME, 100 µm polydimethylsiloxane-coated fibers, Supelco). Samples were preconditioned for 2 h at 80°C, the fiber was equilibrated for 35 min at 80°C and desorbed for 195 s (injection port 260°C) followed by a splitless injection (0.7 min). The Shimadzu GC14A/FID was equipped with a DB-608 capillary column (30 m

length; 0.25 mm i.d.; 0.5 µm film thickness; J&W Scientific, Folsom, USA). The temperature program was used as follows: 3 min at 170°C, 1°C per min to 175°C, 5°C per min to 290°C, held for 5 min. The FID was operated at 300°C.

Chlorophenols were analyzed by HPLC. Alternatively, for the separation of 2,4- and 3,4-DCP and for the analysis of PCP dechlorination, the Shimadzu GC2010 equipped with an electron capture detector was used. The chlorophenols were separated on an Rtx®35 capillary column (see above) and the injector and detector were operated at 260°C and 310°C, respectively. Helium was used as carrier gas and nitrogen as make-up gas. For separation the temperature program was used as follows: initial hold at 100°C (1 min), increase to 156.9°C at a rate of 3.5°C per min (1 min); increase to 190°C at a rate of 3.5°C per min, increase at a rate of 30°C per min to 290°C (final hold 5 min). 3,4,5-Trichloroveratrol (TCV, dissolved in *n*-hexane) was used as internal standard.

References:

1. **Yu NY, Laird MR, Spencer C, Brinkman FS.** 2011. PSORTdb--an expanded, auto-updated, user-friendly protein subcellular localization database for Bacteria and Archaea. Nucleic Acids Res. **39:**D241-244.
2. **Bunge M, Wagner A, Fischer M, Andreesen JR, Lechner U.** 2008. Enrichment of a dioxin-dehalogenating *Dehalococcoides* species in two-liquid phase cultures. Environ. Microbiol. **10:**2670-2683.

Table S2: Dechlorination products formed by strain DCMB5 from different chlorinated dibenzo-*p*-dioxin congeners[a]

Dioxin congener added	Incubation time (days)	Dechlorination products formed (mol%)					
		1,2,3-TrCDD	2,3-DCDD	1,3-DCDD	2-MCDD	1-MCDD	DD
1,2,3,4-TeCDD	1	0.3 ± 0.3	2.6 ± 0.5		0.9 ± 0.1		
	3	0.4 ± 0.2	8.6 ± 0.4	0.8 ± 0.4	2.4 ± 0.3		
1,2,3-TrCDD	4		19.4 ± 8.8	traces	1.8 ± 0.6		
	7		55.8 ± 14.9	traces	5.0 ± 2.6		
1,2,4-TrCDD	2			10.0 ± 6.8	25.4 ± 5.8		
	8			14.4 ± 2.2	65.2 ± 2.6		
2,3-DCDD	8	no products					
1,3-DCDD	4				19.7 ± 1.6		
	7				25.1 ± 7.6		
1,2-DCDD	3				65.3 ± 9.1		
1,4-DCDD	4					12.9 ± 3.1	2.3 ± 0.7
	11					35.6 ± 4.8	19.6 ± 6.4
1-MCDD	4						4.4 ± 0.8
	15						8.7 ± 0.9
2-MCDD	4	no products					

[a]The dibenzo-*p*-dioxin congeners were added to a concentration of 25 µM (except for 1,2-DCDD: 12.5 µM). The concentration of individual congeners is given as mol% of the sum of all congeners detected. The mean values and standard deviations from duplicate or triplicate cultures are given.

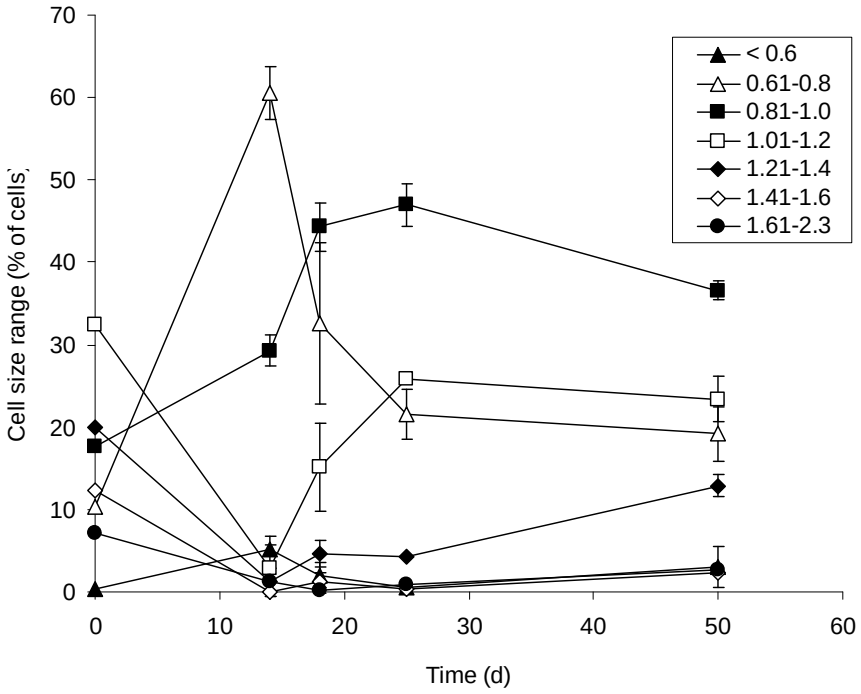

FIG S1: Cell size (µm) distribution during growth with 1,2,3-TCB. Cell cultures were inoculated with 5×10^6 cells ml^{-1}. The cell sizes were determined from phase-contrast images using the AxioVision software (vers. 4.6.3) (Zeiss, Oberkochen, Germany). Approximately 200-350 cells were measured for every data point and culture. The percentage of cells present in the defined cell size ranges (µm) is represented as the mean values and SD of duplicate cultures.

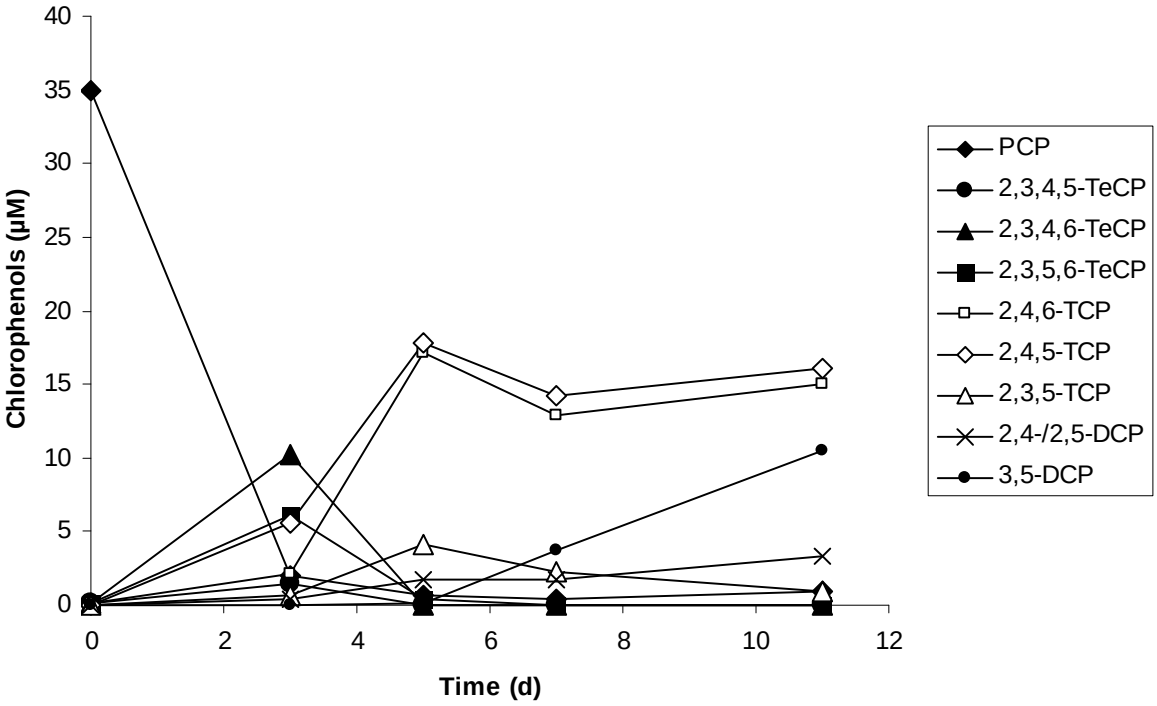

FIG S2: Reductive dechlorination of PCP by strain DCMB5. Mean values of triplicate cultures are shown. PCP, TeCP, TCP, DCP, represent penta-, tetra-, tri-, dichlorophenols, respectively.

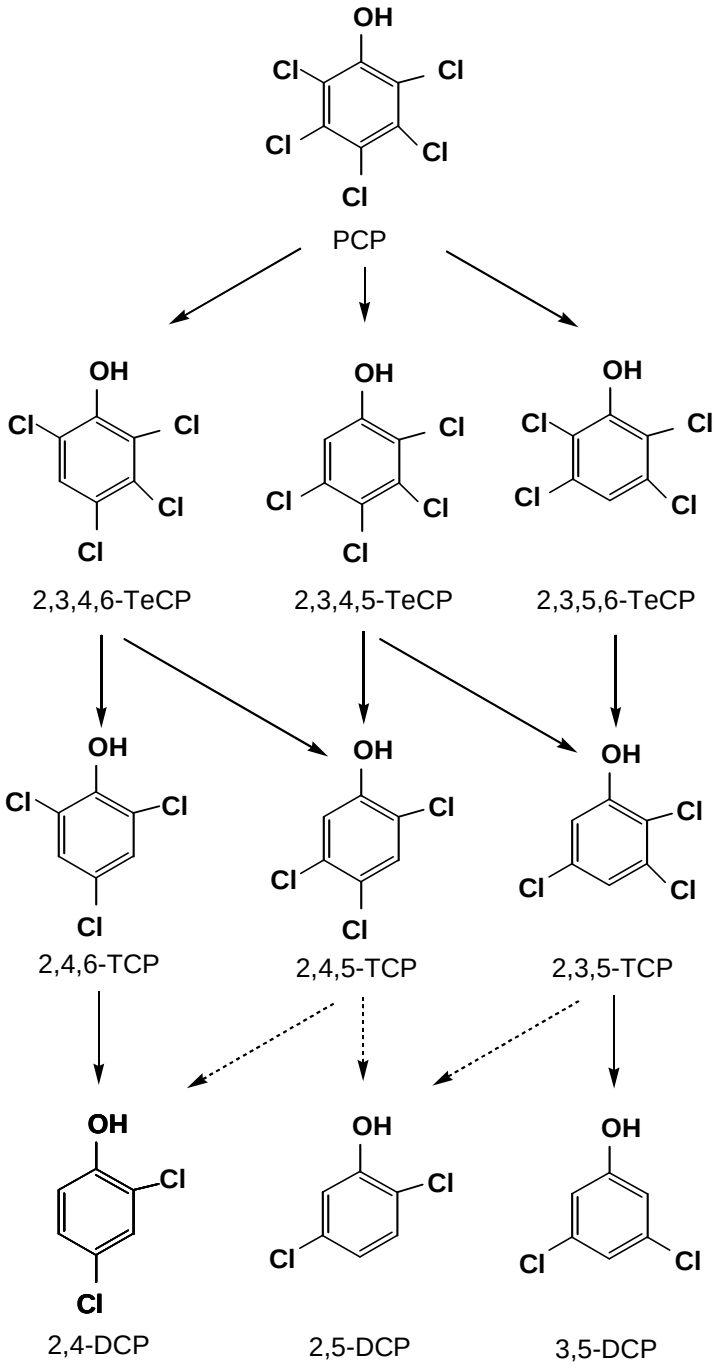

FIG S3: Pathway of reductive dechlorination of PCP by strain DCMB5 leading to 2,4,5-, 2,4,6-TCP, 2,4-and 3,5-DCP as the main products. The dechlorination pathway was confirmed by analysing the dechlorination of the individual intermediates. Major and minor dechlorination routes are represented by solid and broken arrows, respectively.

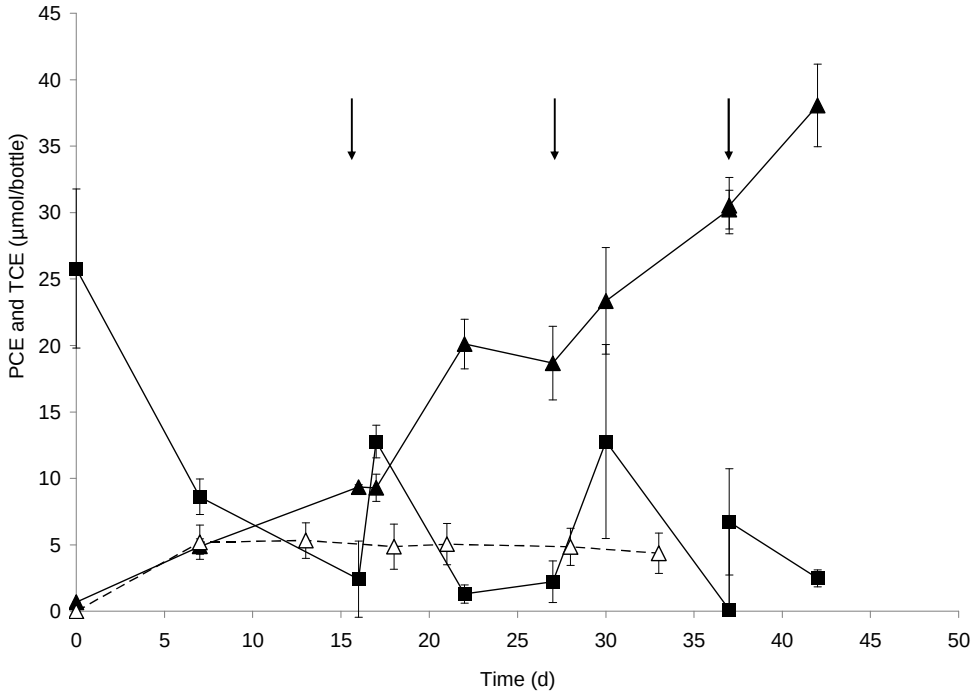

FIG S4 Reductive dechlorination of PCE (■) to TCE (▲). The results (mean values and standard deviations) from two of three replicate cultures and of two abiotic controls (TCE, Δ) are shown. PCE was added at the beginning of the experiment and at the indicated time points (arrows).

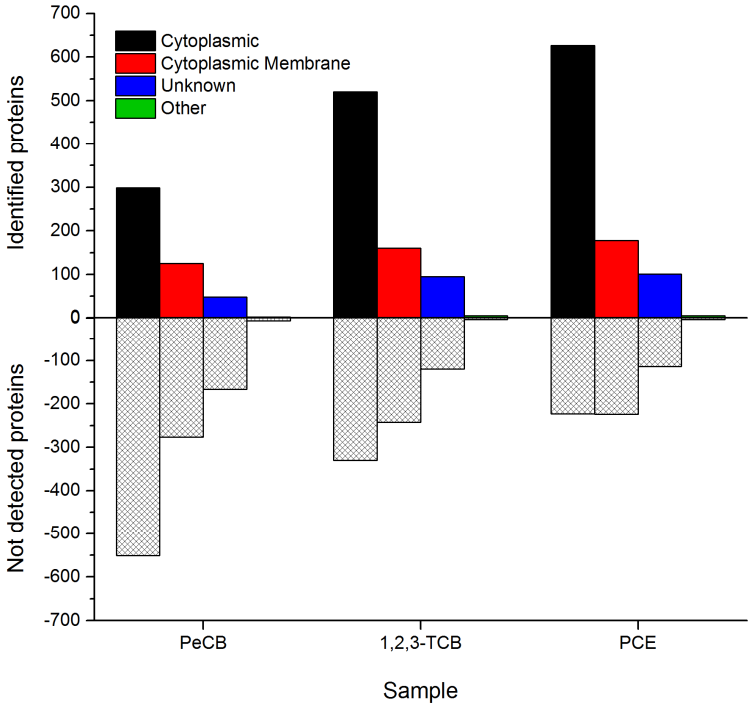

FIG S5: Subcellular localization of proteins identified in cells cultivated with PeCB, 1,2,3-TCB and PCE. The proteins were classified into the different subcellular localizations according to the pSORT database. Rdh proteins are included in the cytoplasmic membrane group. Extracellular and cell wall proteins were combined as other proteins. The combined values of all three replicates are given. The number of undetected proteins refers to the total number of annotated proteins in strain DCMB5.