Supplementary Material to

Functional genotyping of *Sulfurospirillum* spp. in mixed cultures allowed the identification of a new tetrachloroethene reductive dehalogenase

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Additional experimental data

Quantitative PCR for *Sulfurospirillum rdhA* genes. For qPCR, the plasmids pT1P-T_Q, pT1P-D_Q, and pT2R_Q were used as standards (**Table S1**). Linearity and reproducibility of the standard curves was tested using the primers set T1-PTQ-f/-r, T1PDQ-f/-r and T2-RQ-f/-r (**Table S2**). The plasmids containing the target sequence were linearized by digestion for 2 h at 37°C under the following conditions: 10 U/µl of restriction enzyme of *Sca*I (Promega), 1 µl of 10× buffer, 0.2 µl of BSA, 1000 ng/µl of plasmid DNA and the remaining volume of ddH_2O to a final volume of 10 µl. The linearized plasmid was then dephosphorylated with 1 µl of shrimp alkaline phosphatase (Takara Bio Inc., Otsu, Shiga, Japan) and 1 µl of 10× buffer for 1 h at 37°C. The product was finally purified with the Qiagen PCR purification kit according to the instructions and eluted in 50 µl. The DNA was quantified with the NanoDrop spectrophotometer. Gene copy number per µl was calculated using the length (bp) of the reference plasmids, the average molecular weight of a base pair in double-stranded DNA (660 Da) and the obtained concentration in ng/µl according to **Eq. 1**. The qPCR standards were diluted from 10^7 down to 10^1 copies/µl. Standards curves of qPCR for $pceA_{TCE}$, $pceA_{DCE}$ and *rdhA*2 had a linear range between 10^2 and 10^7 gene copies/µl.

Eq. 1: $$\text{copie}/\mu\text{l} = \frac{\frac{\text{DNA concentration (ng}/\mu\text{l)}}{10^9}}{\text{Molecular Weight } (\frac{\text{g}}{\text{mol}})} \times \text{Avogadro's number}$$

Reactions for qPCR were prepared as follows: per 10 µl reaction volume, 5.0 µl of KAPA SYBR® FAST qPCR mix (KAPAbiosystems, Boston, Massachusetts, United States), 0.2 µl of each primer at 10 µM, and 2.1 µl of ddH_2O water. A volume of 2.5 µl DNA template (standards and samples) was added to each tube. Samples and standards were always measured in triplicates. Primers specifically developed for this purpose are listed in **Table S2**.

Real-time PCR was performed in a RotorGene RG3000 real-time PCR machine (Corbett Research, Sydney, Australia) in the 72-well rotor. The thermocycling program for qPCR was as follows : 15 min 94°C initial denaturation, followed by 50 cycles of 30 s at 94°C, 20 s at 60°C or 62°C depending on the target gene, and 30 s at 72°C, after which data acquisition took place using the SYBR detection channel. Finally, a melting curve ranging from 72 to 99°C was recorded with 1°C increments and a hold of 5 s. For each run standards were included and all samples were calculated with this standard curve.

The Rotor-Gene Analysis Software V6.0 was used for analysis of qPCR data. Threshold fluorescence levels were set to the lowest level that minimized error in standard curves, typically at values between 0.1 and 0.15. Slopes and R^2 values of semi-logarithmic regression curves of the standards were routinely around -3.6 ± 0.4 and > 0.98, respectively. The comparison of the values obtained by qPCR and by T-RFLP showed that most samples had relatively the same proportions of the different *rdhA* genes with both techniques (**Table 2** in the paper). Therefore we can reasonably consider the *rdhA*-specific T-RFLP analysis as a semi-quantitative analysis.

Table S1. Bacterial strains and plasmids used in this work.

Bacteria/consortia	**Details**	**References**
SL2-PCEb	Bacterial consortium containing *Sulfurospirillum* spp. and dechlorinating PCE to *cis*-DCE in a stepwise manner	(1, 2)
SL2-PCEc	Bacterial consortium selected from SL2-PCEb displaying PCE to TCE dechlorination	This study
SL2-TCE	Bacterial consortium selected from SL2-PCEb on TCE and displaying PCE to *cis*-DCE dechlorination	This study
Sulfurospirillum multivorans	DSM 12446	(3)
Sulfurospirillum halorespirans	strain PCE-M2, DSM 13726	(4)
Escherichia coli DH5α	genotype: F^- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17(r_K^- m_K^+), λ–	Laboratory strain
Plasmids	**Details**	**References**
pGEM-T Easy	Cloning vector for PCR products	Promega
pT1P-T_S	pGEM-T harboring the complete *pceAB*$_{TCE}$ genes identified in SL2-PCEc	This study
pT1P-D_S	pGEM-T harboring the complete *pceAB*$_{DCE}$ genes identified in SL2-TCE	This study
pT2R$_S$	pGEM-T harboring the complete *rdhAB2* genes identified in SL2-PCEc and SL2-TCE	This study
pT1P-T_Q	pGEM-T harboring a fragment of type-1 *pceA*$_{TCE}$ used as qPCR reference plasmid	This study
pT1P-D_Q	pGEM-T harboring a fragment of type-1 *pceA*$_{DCE}$ used as qPCR reference plasmid	This study
pT2R$_Q$	pGEM-T harboring a fragment of type-2 *rdhA* used as qPCR reference plasmid	This study

Table S2. Oligonucleotides used in the work.

Primer name	Target gene	Primer sequence 5'→ 3'
8f-FAM	16S rRNA	AGAGTTTGATCMTGGCTCAG[a]
8f		AGAGTTTGATCCTGGCTCAG
518r		ATTACCGCGGCTGCTGG
1492rm		GNTACCTTGTTACGACTT[a]
Sul-16S-if		CGAAGGCGATCTACTGGAAC
Sul-16S-ir		GTTCCAGTAGATCGCCTTCG
Sul-rdhA-f-FAM	*Sul-rdhA*	TTRGTRGGTRTTGCAAGATT[a]
Sul-rdhA-f		TTRGTRGGTRTTGCAAGATT[a]
Sul-rdhA-r		CTTGTCCTAAACCTGCTTC
T1-PTQ-f	$pceA_{TCE}$	CTTTGGAGGTAACTTTGGAGGTTA
T1-PTQ-r		CTTTAGGCCAAGATTGTTCATCT
T1-PDQ-f	$pceA_{DCE}$	GTAACTATACCAGCTGACGTACC
T1-PDQ-r		CATAGCGATACCTGCAACGA
T1-*pceAB*-f	$pceA_{TCE}$/$pceA_{DCE}$	CAAGAAGGTTTGAATACCACTGT
T1-*pceAB*-r		ATGATGTAAACCCTACTTTATGC
T1-*pceAB*-is1-f		ACGTATGGCAGGTGCTGATT
T1-*pceAB*-is2-f		GCCTATCGACTTTGGAGTAACA
T1-*pceAB*-is3-r		ATAAGCCATTCAACGCCATC
T2-RQ-f	*Sul-rdhA*2	GCCTGAGGATGATAATAACT
T2-RQ-r		CATATCATGCGCCACCATAC
T2-*rdhAB2*-f		CAGGACTTGGACTCTACACAGC
T2-*rdhAB2*-r		CGAGTTTGTTCTTCATCATCTGC
T2-*rdhA*2-is-f		ATCTCAGAAGGGCCACAATC
T2-*rdhA*2-is-r		AGTGTCCACGCAGCTGATTC
SP6	pGEM-T Easy	ATTTAGGTGACACTATAGAA
T7		TAATACGACTCACTATAGGG

[a] Abbreviations of degenerate nucleotides: M = A/C; N = A/C/G/T; R = A/G.

Table S3. *Sulfurospirillum* 16S rRNA gene fingerprinting challenge.

Strain	Database ref.	*Hae*III site	Detected *rdhA* genes
S. multivorans DSM12446	X82931	254 [c]	2
S. halorespirans PCE-M2	NR_028771	254	2
S. deleyianium DSM6946 [a]	NR_074378	254	none
S. barnesii SES-3 [b]	NR_028692	254	none
S. cavolei Phe91	NR_041392	254 [c]	n.a.
S. arsenophilum MIT-13	NR_044806	254 [c]	n.a.
S. archachonense F1F6	NR_026408	1175 [c]	n.a.
S. carboxydovorans MV	AY740528	1175	n.a.
Sulfurospirillum sp. JPD-1	AY189928	256 [c]	n.a.
Sulfurospirillum sp. Am-N	AF357198	256	n.a.

[a] *S. deleyianium* genome contains 3 copies of 16S rRNA gene, all of them harboring a *Hae*III site at 254 bp.
[b] *S. barnesii* genome contains 2 copies of 16S rRNA gene, all of them harboring a *Hae*III site at 254 bp.
[c] A few nucleotides were missing at the 5'-end of these sequences. The *Hae*III site was obtained after filling up the gaps with the number of nucleotides found in the respective closest relative sequence.
n.a. not available

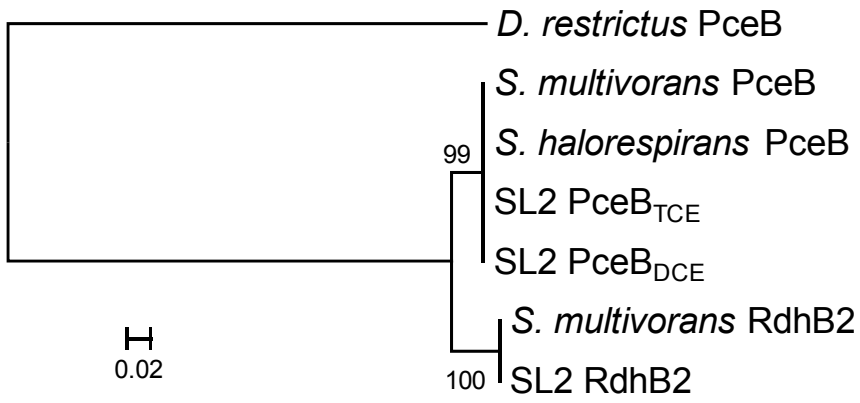

Figure S1. Sequence likelihood analysis showing the relationship of newly identified RdhB from SL2 consortia to known RdhB sequences. The neighbor-joining method of ClustalX was used to build the tree, including 100X bootstrap values. All sequences used in the alignment had a similar length, *S. multivorans* PceB (Genbank AAC60789), *S. halorespirans* PceB (Genbank AAG46195), and *S. multivorans* RdhB2 (T. Schubert, University of Jena, personal communication). The tree was rooted with PceB of *Dehalobacter restrictus* (Genbank CAD62441). The scale bar represents 2% of sequence divergence.

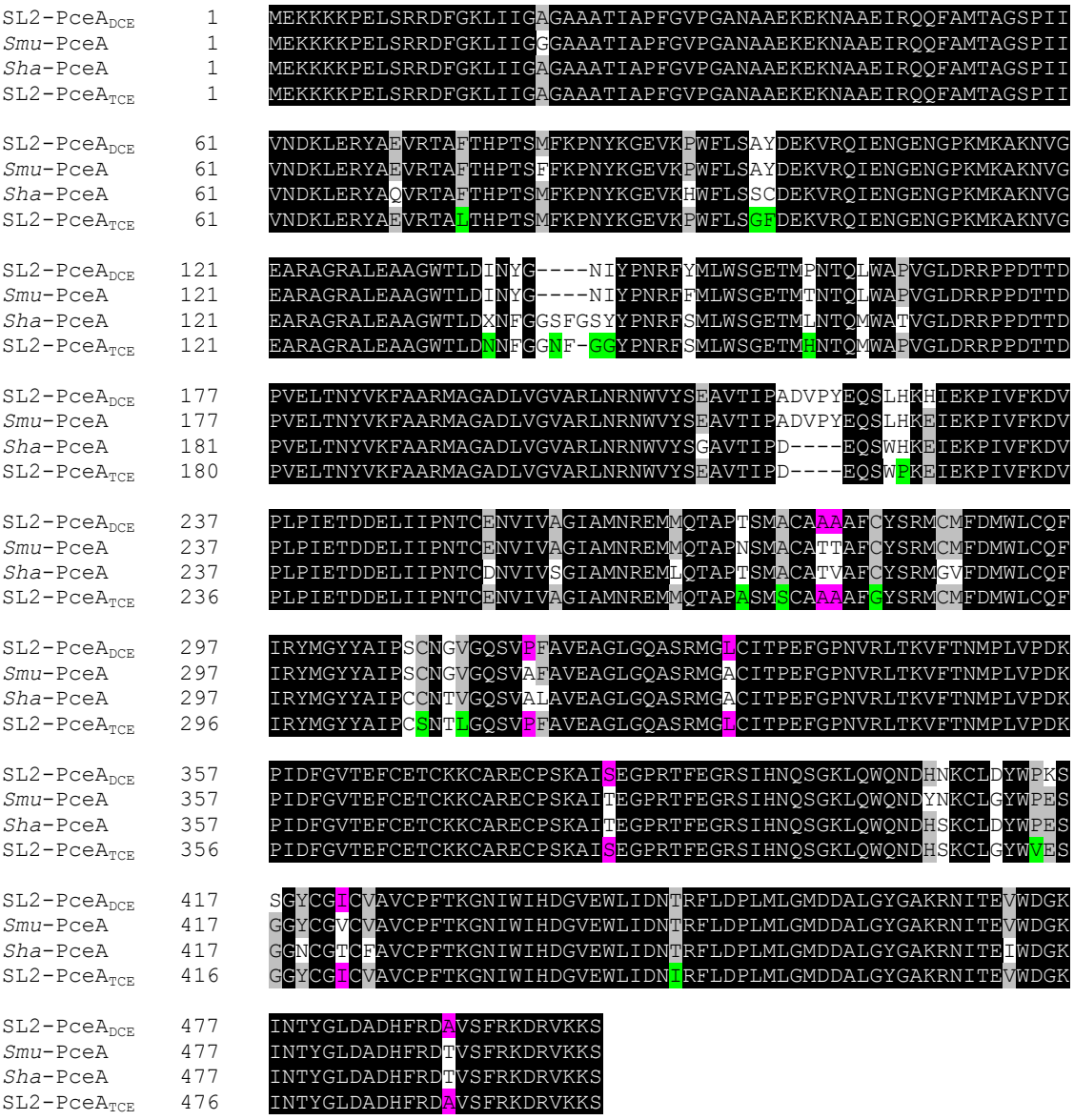

Figure S2. Sequence alignment of type-1 PceA proteins from SL2 with *S. multivorans* PceA (*Smu*-PceA) and *S. halorespirans* PceA (*Sha*-PceA). The sequence alignment was done in ClustalX. Black-shaded amino acids are fully conserved among all four proteins, grey-shaded ones are positions conserved in three out of four sequences, and the 16 green-shaded amino acids are unique to SL2-PceA$_{TCE}$. Pink-shaded amino acids are only conserved in both SL2-PceA sequences. Noteworthy are two compensatory insertion/deletion (indel) regions, the first one in *Smu*-PceA and SL2-PceA$_{DCE}$, and the second one almost fully conserved in *Sha*-PceA and SL2-PceA$_{TCE}$.

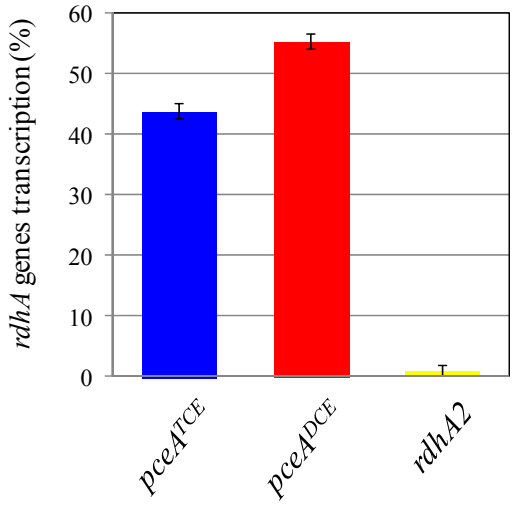

Figure S3. Transcription of *rdhA* genes using the dedicated T-RFLP analysis on cDNA from the consortium SL2-PCEb collected at a single time point during PCE dechlorination (approximately half-way).

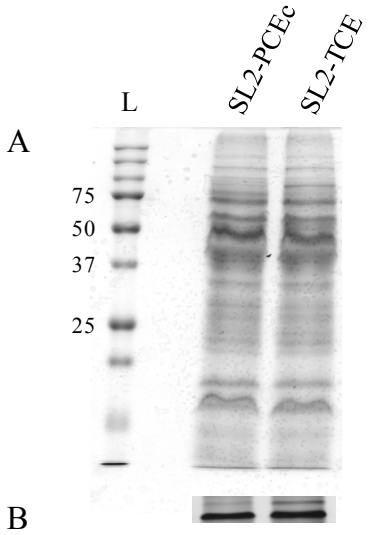

Figure S4. PceA detection in SL2-PCEc and SL2-TCE crude extracts. Crude extracts of SL2-PCEc and SL2-TCE were obtained after cultivation on PCE and TCE, respectively, and analyzed by SDS-PAGE followed by (A) Coomassie staining and, (B) Western blot analysis with *S. multivorans* anti-PceA serum. Both enzyme forms (with or without the Tat signal peptide attached, respectively) are visible. L: molecular mass ladder (in kDa).

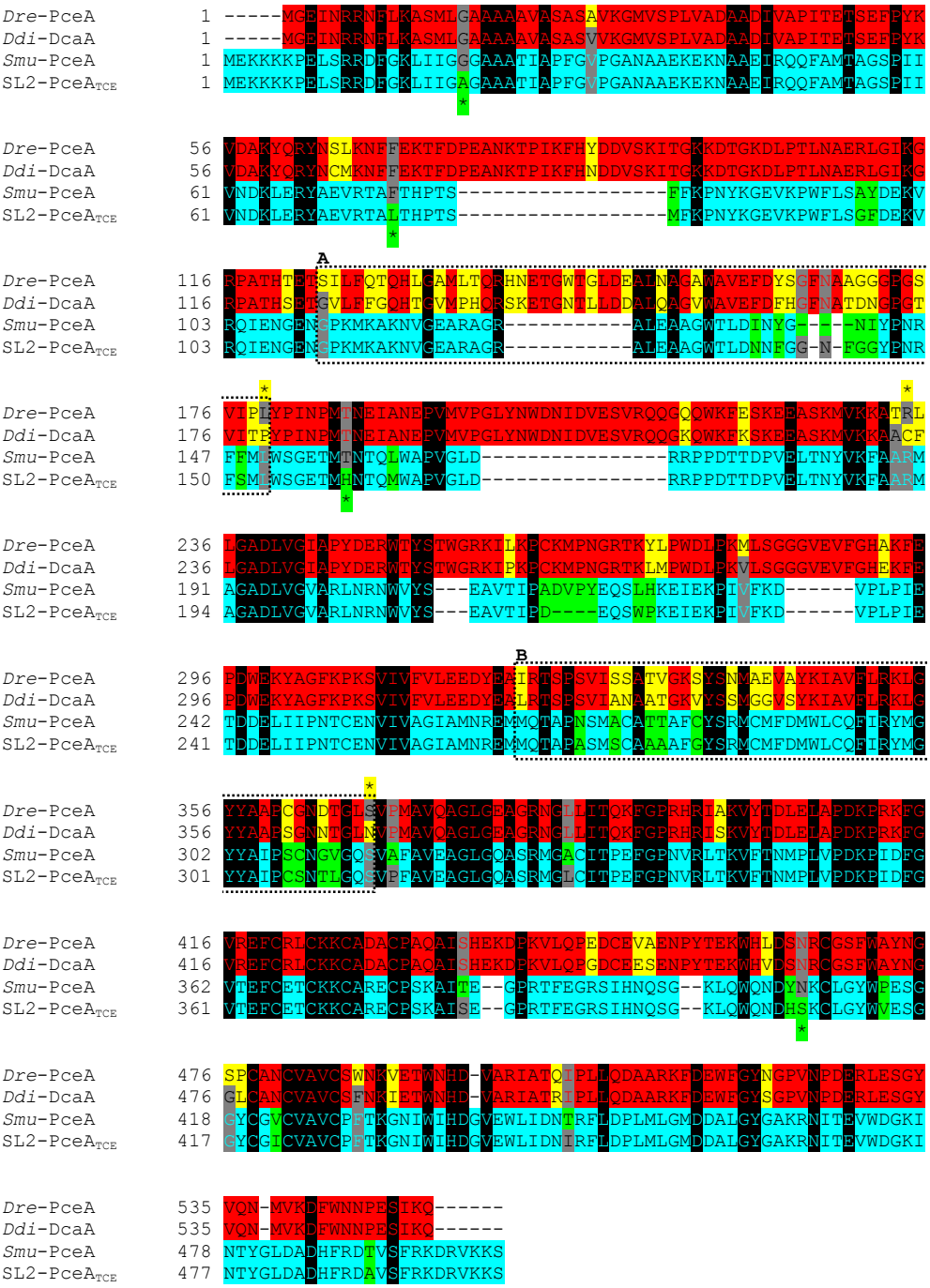

Figure S5. Sequence pair alignment of *Dehalobacter restrictus* PceA (*Dre*-PceA) and *Desulfitobacterium dichloroeliminans* DcaA (*Ddi*-DcaA) (top two sequences), with *Sulfurospirillum multivorans* PceA (*Smu*-PceA) and $PceA_{TCE}$ from SL2 consortia (SL2-PceATCE) (bottom two sequences). Fully conserved amino acids among the four sequences (core) are shaded in black. Additional amino acids conserved in the top two sequences are shaded or written in red, those in the bottom two sequences in cyan. Amino acids conserved in three out of four sequences are shaded in grey. Amino acid substitutions between the top two sequences are shaded in yellow and in green between the bottom two sequences. Dashed boxes A and B depict the two regions identified by Marzorati *et al*. (5) in which more than half of the 62 amino acid substitutions between *Dre*-PceA and *Ddi*-DcaA are located. Yellow or green shaded stars indicate amino acids that are unique to *Ddi*-DcaA or SL2-$PceA_{TCE}$, respectively.

References

1. **Maillard J, Charnay MP, Regeard C, Rohrbach-Brandt E, Rouzeau-Szynalski K, Rossi P, and Holliger C.** 2011. Reductive dechlorination of tetrachloroethene by a stepwise catalysis of different organohalide respiring bacteria and reductive dehalogenases. Biodegradation **22:**949-960.
2. **Rouzeau-Szynalski K, Maillard J, and Holliger C.** 2011. Frequent concomitant presence of *Desulfitobacterium* spp. and "*Dehalococcoides*" spp. in chloroethene-dechlorinating microbial communities. Appl Microbiol Biotechnol **90:**361-368.
3. **Scholz-Muramatsu H, Neumann A, Messmer M, Moore E, and Diekert G.** 1995. Isolation and characterization of *Dehalospirillum multivorans* gen. nov., sp. nov., a tetrachloroethene-utilizing, strictly anaerobic bacterium. Arch Microbiol **163:**48-56.
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