## 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<sub>Q</sub>, pT1P-D<sub>Q</sub>, and pT2R<sub>Q</sub> 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 *ScaI* (Promega), 1 µl of 10× buffer, 0.2 µl of BSA, 1000 ng/µl of plasmid DNA and the remaining volume of ddH<sub>2</sub>O 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<sup>7</sup> down to 10<sup>1</sup> copies/µl. Standards curves of qPCR for *pceA*<sub>TCE</sub>, *pceA*<sub>DCE</sub> and *rdhA2* had a linear range between 10<sup>2</sup> and 10<sup>7</sup> gene copies/µl.

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

Reactions for qPCR were prepared as follows: per 10  $\mu$ l reaction volume, 5.0  $\mu$ l of KAPA SYBR® FAST qPCR mix (KAPAbiosystems, Boston, Massachusetts, United States), 0.2  $\mu$ l of each primer at 10  $\mu$ M, and 2.1  $\mu$ l of ddH<sub>2</sub>O water. A volume of 2.5  $\mu$ l 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  $-3.6 \pm 0.4$  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.

Bacteria/consortia	Details	References
SL2-PCEb	Bacterial consortium containing <i>Sulfurospirillum</i> spp. and dechlorinating PCE to <i>cis</i> -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 <i>cis</i> -DCE dechlorination	This study
Sulfurospirillum multivorans	DSM 12446	(3)
Sulfurospirillum halorespirans	strain PCE-M2, DSM 13726	(4)
<i>Escherichia coli</i> DH5α	genotype: F <sup>-</sup> endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG $\Phi$ 80d <i>lacZ</i> $\Delta$ M15 $\Delta$ ( <i>lacZYA-argF</i> )U169, hsdR17(r <sub>K</sub> <sup>-</sup> m <sub>K</sub> <sup>+</sup> ), $\lambda$ -	Laboratory strain
Plasmids	Details	References
<b>Plasmids</b> pGEM-T Easy	Details Cloning vector for PCR products	<b>References</b> Promega
<b>Plasmids</b> pGEM-T Easy pT1P-T <sub>s</sub>	Details         Cloning vector for PCR products         pGEM-T harboring the complete <i>pceAB</i> <sub>TCE</sub> genes         identified in SL2-PCEc	References Promega This study
Plasmids pGEM-T Easy pT1P-T <sub>s</sub> pT1P-D <sub>s</sub>	Details         Cloning vector for PCR products         pGEM-T harboring the complete <i>pceAB</i> <sub>TCE</sub> genes identified in SL2-PCEc         pGEM-T harboring the complete <i>pceAB</i> <sub>DCE</sub> genes identified in SL2-TCE	References Promega This study This study
PlasmidspGEM-T EasypT1P-TspT1P-DspT2Rs	DetailsCloning vector for PCR productspGEM-T harboring the complete <i>pceAB</i> TCE genes identified in SL2-PCEcpGEM-T harboring the complete <i>pceAB</i> DCE genes identified in SL2-TCEpGEM-T harboring the complete <i>rdhAB2</i> genes identified in SL2-PCEc and SL2-TCE	ReferencesPromegaThis studyThis studyThis study
Plasmids pGEM-T Easy pT1P-T <sub>s</sub> pT1P-D <sub>s</sub> pT2R <sub>s</sub> pT1P-T <sub>Q</sub>	DetailsCloning vector for PCR productspGEM-T harboring the complete pceABTCE genes identified in SL2-PCEcpGEM-T harboring the complete pceABDCE genes identified in SL2-TCEpGEM-T harboring the complete rdhAB2 genes identified in SL2-PCEc and SL2-TCEpGEM-T harboring a fragment of type-1 pceATCE used as qPCR reference plasmid	ReferencesPromegaThis studyThis studyThis studyThis study
Plasmids pGEM-T Easy pT1P-Ts pT1P-Ds pT2Rs pT1P-TQ pT1P-DQ	DetailsCloning vector for PCR productspGEM-T harboring the complete pceABTCE genes identified in SL2-PCEcpGEM-T harboring the complete pceABDCE genes identified in SL2-TCEpGEM-T harboring the complete rdhAB2 genes identified in SL2-PCEc and SL2-TCEpGEM-T harboring a fragment of type-1 pceATCE used as qPCR reference plasmidpGEM-T harboring a fragment of type-1 pceADCE used as qPCR reference plasmid	ReferencesPromegaThis studyThis studyThis studyThis studyThis study

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

Primer name	Target gene	Primer sequence $5' \rightarrow 3'$
8f-FAM		AGAGTTTGATCMTGGCTCAG <sup>a</sup>
8f		AGAGTTTGATCCTGGCTCAG
518r		ATTACCGCGGCTGCTGG
1492rm	105 IKNA	GNTACCTTGTTACGACTT <sup>a</sup>
Sul-16S-if		CGAAGGCGATCTACTGGAAC
Sul-16S-ir		GTTCCAGTAGATCGCCTTCG
Sul-rdhA-f-FAM		TTRGTRGGTRTTGCAAGATT <sup>a</sup>
Sul-rdhA-f	Sul-rdhA	TTRGTRGGTRTTGCAAGATT <sup>a</sup>
Sul-rdhA-r		CTTGTCCTAAACCTGCTTC
T1-PTQ-f		CTTTGGAGGTAACTTTGGAGGTTA
T1-PTQ-r	pceA <sub>TCE</sub>	CTTTAGGCCAAGATTGTTCATCT
T1-PDQ-f	4	GTAACTATACCAGCTGACGTACC
T1-PDQ-r	pceA <sub>DCE</sub>	CATAGCGATACCTGCAACGA
T1-pceAB-f		CAAGAAGGTTTGAATACCACTGT
T1-pceAB-r		ATGATGTAAACCCTACTTTATGC
T1-pceAB-is1-f	pceA <sub>TCE</sub> /pceA <sub>DCE</sub>	ACGTATGGCAGGTGCTGATT
T1-pceAB-is2-f		GCCTATCGACTTTGGAGTAACA
T1-pceAB-is3-r		ATAAGCCATTCAACGCCATC
T2-RQ-f		GCCTGAGGATGATAATAACT
T2-RQ-r	Sul-rdhA2	CATATCATGCGCCACCATAC
T2- <i>rdhAB2</i> -f		CAGGACTTGGACTCTACACAGC
T2- <i>rdhAB2</i> -r		CGAGTTTGTTCTTCATCATCTGC
T2-rdhA2-is-f		ATCTCAGAAGGGCCACAATC
T2-rdhA2-is-r		AGTGTCCACGCAGCTGATTC
SP6		ATTTAGGTGACACTATAGAA
Τ7	poem-i easy	TAATACGACTCACTATAGGG

 Table S2. Oligonucleotides used in the work.

<sup>a</sup> Abbreviations of degenerate nucleotides: M = A/C; N = A/C/G/T; R = A/G.

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

Table S3. Sulfurospirillum 16S rRNA gene fingerprinting challenge.

<sup>a</sup> S. deleyianium genome contains 3 copies of 16S rRNA gene, all of them harboring a HaeIII site at 254 bp.

<sup>b</sup> *S. barnesii* genome contains 2 copies of 16S rRNA gene, all of them harboring a *Hae*III site at 254 bp.

<sup>c</sup> 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 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.

SL2-PceA <sub>DCE</sub> Smu-PceA	1 1	MEKKKKPELSRRDFGKLIIG <mark>A</mark> GAAATIAPFGVPGANAAEKEKNAAEIRQQFAMTAGSPII MEKKKKPELSRRDFGKLIIG <mark>G</mark> GAAATIAPFGVPGANAAEKEKNAAEIRQQFAMTAGSPII
SL2-PCEA	1	MEKKKKPELSRRDFGKLIIG <mark>A</mark> GAAATIAPFGVPGANAAEKEKNAAEIRQQFAMTAGSPII MEKKKKPELSRRDFGKLIIG <mark>A</mark> GAAATIAPFGVPGANAAEKEKNAAEIRQQFAMTAGSPII
SL2-PceA <sub>DCE</sub> Smu-PceA	61 61	VNDKLERYAE <mark>VRTAE</mark> THPTSMFKPNYKGEVK <mark>P</mark> WFLSAYDEKVRQIENGENGPKMKAKNVG VNDKLERYAEVRTAETHPTSFFKPNYKGEVKPWFLSAYDEKVROIENGENGPKMKAKNVG
Sha-PceA	61	VNDKLERYA <mark>Q</mark> VRTA <mark>F</mark> THPTS <mark>M</mark> FKPNYKGEVK <mark>H</mark> WFLS <mark>SC</mark> DEKVRQIENGENGPKMKAKNVG
SL2-PCeA <sub>TCE</sub>	61	VNDKLERYA <mark>B</mark> VRTAL <mark>THPTSM</mark> FKPNYKGEVK <mark>P</mark> WFLS <mark>GF</mark> DEKVRQLENGENGPKMKAKNVG
$SL2-PceA_{DCE}$	121	EARAGRALEAAGWTLDINYGNIYPNRFYMLWSGETMPNTQIWAPVGLDRRPPDTTD
Smu-PceA	121	EARAGRALEAAGWTLDINYGNIYPNRFFMLWSGETMTNTQLWAPVGLDRRPPDTTD
Sha-PceA	121	EARAGRALEAAGWTLDXNFGGSFGSYYPNRFSMLWSGETMLNTOMWATVGLDRRPPDTTD
SL2-PceA <sub>TCE</sub>	121	EARAGRALEAAGWTLD <mark>NN</mark> E <mark>'GGNE'-GG</mark> YPNRE <mark>SMLWSGETMH</mark> NTQMWAPVGLDRRPPD'I'I'D
$SL2-PceA_{DCE}$	177	PVELTNYVKFAARMAGADLVGVARLNRNWVYS <mark>E</mark> AVTIP <mark>ADVPY</mark> EQS <mark>LHKH</mark> IEKPIVFKDV
Smu-PceA	177	PVELTNYVKFAARMAGADLVGVARLNRNWVYSEAVTIPADVPYEQSLHKEIEKPIVFKDV
Sha-PceA	181	PVELTNYVKFAARMAGADLVGVARLNRNWVYSGAVTIPDEQSWHKEIEKPIVFKDV
SL2-PceA <sub>TCE</sub>	180	PVELTNYVKFAARMAGADLVGVARLNRNWVYSEAVTIPDEQSWPKEIEKPIVFKDV
$SL2-PceA_{DCE}$	237	PLPIETDDELIIPNTC <mark>E</mark> NVIV <mark>A</mark> GIAMNREM <mark>M</mark> QTAPT <mark>SMACA<mark>AA</mark>AFCYSRM<mark>CM</mark>FDMWLCQF</mark>
Smu-PceA	237	PLPIETDDELIIPNTC <mark>E</mark> NVIVA <mark>GIAMNREMM</mark> QTAP <mark>NSM</mark> ACATTAFCYSRMCMFDMWLCQF
Sha-PceA	237	PLPIETDDELIIPNTC <mark>D</mark> NVIV <mark>S</mark> GIAMNREM <mark>LQTAP</mark> TSM <mark>A</mark> CA <mark>TV</mark> AF <mark>C</mark> YSRM <mark>GV</mark> FDMWLCQF
$SL2-PceA_{TCE}$	236	PLPIETDDELIIPNTC <mark>E</mark> NVIV <mark>A</mark> GIAMNREM <mark>M</mark> QTAP <mark>A</mark> SM <mark>S</mark> CA <mark>AA</mark> AF <mark>G</mark> YSRM <mark>CM</mark> FDMWLCQF
SL2-PceA <sub>DCE</sub>	297	IRYMGYYAIP <mark>SC</mark> NGVGQSV <mark>PF</mark> AVEAGLGQASRMG <mark>L</mark> CITPEFGPNVRLTKVFTNMPLVPDK
Smu-PceA	297	IRYMGYYAIP <mark>SC</mark> N <mark>GV</mark> GQSV <mark>AF</mark> AVEAGLGQASRMG <mark>A</mark> CITPEFGPNVRLTKVFTNMPLVPDK
Sha-PceA	297	IRYMGYYAIP <mark>CC</mark> N <mark>TV</mark> GQSV <mark>AL</mark> AVEAGLGQASRMG <mark>A</mark> CITPEFGPNVRLTKVFTNMPLVPDK
$SL2-PceA_{TCE}$	296	IRYMGYYAIP <mark>CS</mark> N <mark>TL</mark> GQSV <mark>PF</mark> AVEAGLGQASRMG <mark>L</mark> CITPEFGPNVRLTKVFTNMPLVPDK
SL2-PceA <sub>DCE</sub>	357	PIDFGVTEFCETCKKCARECPSKAI <mark>S</mark> EGPRTFEGRSIHNQSGKLQWQND <mark>HN</mark> KCL <mark>D</mark> YW <mark>PK</mark> S
Smu-PceA	357	PIDFGVTEFCETCKKCARECPSKAI <mark>T</mark> EGPRTFEGRSIHNQSGKLQWQND <mark>YN</mark> KCL <mark>G</mark> YW <mark>PE</mark> S
Sha-PceA	357	PIDFGVTEFCETCKKCARECPSKAI <mark>T</mark> EGPRTFEGRSIHNQSGKLQWQND <mark>HS</mark> KCL <mark>D</mark> YW <mark>PE</mark> S
$SL2-PceA_{TCE}$	356	PIDFGVTEFCETCKKCARECPSKAI <mark>S</mark> EGPRTFEGRSIHNQSGKLQWQND <mark>HS</mark> KCL <mark>G</mark> YW <mark>VE</mark> S
SL2-PceA <sub>DCE</sub>	417	SGYCG <mark>I</mark> CVAVCPFTKGNIWIHDGVEWLIDN <mark>T</mark> RFLDPLMLGMDDALGYGAKRNITE <mark>V</mark> WDGK
Smu-PceA	417	G <mark>GY</mark> CG <mark>VC</mark> VAVCPFTKGNIWIHDGVEWLIDN <mark>T</mark> RFLDPLMLGMDDALGYGAKRNITE <mark>V</mark> WDGK
Sha-PceA	417	G <mark>GNCGTCF</mark> AVCPFTKGNIWIHDGVEWLIDN <mark>T</mark> RFLDPLMLGMDDALGYGAKRNITE <mark>I</mark> WDGK
$SL2-PceA_{TCE}$	416	G <mark>GYCG<mark>IC</mark>VAVCPFTKGNIWIHDGVEWLIDN<mark>I</mark>RFLDPLMLGMDDALGYGAKRNITE<mark>V</mark>WDGK</mark>
SL2-PceA <sub>DCE</sub>	477	INTYGLDADHFRD <mark>A</mark> VSFRKDRVKKS
Smu-PceA	477	INTYGLDADHFRD <mark>T</mark> VSFRKDRVKKS
Sha-PceA	477	INTYGLDADHFRD <mark>T</mark> VSFRKDRVKKS
$SL2-PceA_{TCE}$	476	INTYGLDADHFRD <mark>A</mark> VSFRKDRVKKS

**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<sub>TCE</sub>. 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<sub>DCE</sub>, and the second one almost fully conserved in *Sha*-PceA and SL2-PceA<sub>TCE</sub>.



**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).

Dre-PceA Ddi-DcaA Smu-PceA SL2-PceA <sub>TCE</sub>	1MCEINRRNELKASMLGAAAAAVASASAVKGMVSPLVADAADIVAPITETSEFEYK 1MCEINRRNELKASMLGAAAAAVASASVVKGMVSPLVADAADIVAPITETSEFEYK 1 MEKKKKPELSRRDEGKLIIGGGAAATIAPFG PGANAAEKEKNAAEIRQQFAMTAGSPII 1 MEKKKKPELSRRDEGKLIIGAGAAATIAPFG PGANAAEKEKNAAEIRQQFAMTAGSPII 7
Dre-PceA Ddi-DcaA Smu-PceA SL2-PceA <sub>TCE</sub>	56 VDAKYORYNSLKNEFEKTEDPEANKTPIKEHYDDVSKITCKKDTGKDLPTINAERLGIKG 56 VDAKYORYNCMKNEFEKTEDPEANKTPIKEHNDDVSKITGKKDTGKDLPTINAERLGIKG 61 VNDKLERYAEVRTAETHPTSFEKPNYKGEVKPWFLSAYDEKV 61 VNDKLERYAEVRTALTHPTSMEKPNYKGEVKPWFLSGFDEKV *
Dre-PceA Ddi-DcaA Smu-PceA SL2-PceA <sub>TCE</sub>	A 116 RPATH TETSILFQTOH LEAMITOR HNETGWIGDLE RINAGADAVEFDYSGENAAGGGEGS 116 RPATH SILFQT FOR TOWN PHOR SKETCHILDDAL OR WAVEFDFHGENATDNGEGT 103 RQIENCEN PKMKAKNVCEARAGEALEAAGMTLDNNFGNIYENR 103 RQIENCEN PKMKAKNVCEARAGEALEAAGMTLDNNFGG-N-FGGYENR
Dre-PceA Ddi-DcaA Smu-PceA SL2-PceA <sub>TCE</sub>	* 176 VIPLYPINPMTNEIANE FVMVPGLYNWDNIDVESVRQQGQQWKEESKEEASKMVKKETRL 176 VITEYPINPMTNEIANE FVMVPGLYNWDNIDVESVRQQGQQWKEKSKEEASKMVKKEACF 147 FFMIWSGETMTNTQLWAPVGLDRRPPDTTDPVELTNYVKFAARM 150 FSMIWSGETMHNTQMWAPVGLDRRPPDTTDPVELTNYVKFAARM *
Dre-PceA Ddi-DcaA Smu-PceA SL2-PceA <sub>TCE</sub>	236 LGADLVGIAPYDERWTYSTWGRKILKECKMPNGRTKYLPWDIEKMLSGGGVEVFGHAKTE 236 LGADLVGIAPYDERWTYSTWGRKIPKECKMPNGRTKLMPWDIEKVLSGGGVEVFGHEKFE 191 AGADLVGVARLNRNWVYSEAVTIPADVPYEQSLHKEIEKEIVFKDVPLPIE 194 AGADLVGVARLNRNWVYSEAVTIPDEQSWPKEIEKEIVFKDVPLPIE
Dre-PceA Ddi-DcaA Smu-PceA SL2-PceA <sub>TCE</sub>	B           296         PDWBKYAGFKPKSVIV FVLEEDYB AIRASS SVISS TVGRSVSNMAEVAYKIAV BLEKLG           296         PDWBKYAGFKPKSVIV FVLEEDYB AIRASSVISS TVGRSVSNMAEVAYKIAV BLEKLG           291         TDDBLIIPNTCENVIVAGIAMNRBMMQTAPNSMACATTAFCYSRVCMFDMWLCQFIRYMG           241         TDDBLIIPNTCENVIVAGIAMNRBMMQTAPASMSCAAAAFGYSRVCMFDMWLCQFIRYMG
Dre-PceA Ddi-DcaA Smu-PceA SL2-PceA <sub>TCE</sub>	356       YYAAF CONDTGLSVP MAVQAGLGEAGENGLLIT OKEGPRHRIA KVYTDLELAPDKPRKEG         356       YYAAF SONNTGLNVP MAVQAGLGEAGENGLLIT OKEGPRHRIS KVYTDLELAPDKPRKEG         302       YYAIPSONGVGQEVAFAVEAGLGOASRMGACIT PE FGPNVRLTKVFTNMPLVPDKPIDFG         301       YYAIP CSNTLGQEVPFAVEAGLGOASRMGLCIT PE FGPNVRLTKVFTNMPLVPDKPIDFG
Dre-PceA Ddi-DcaA Smu-PceA SL2-PceA <sub>TCE</sub>	416       VREFORLSKKCADACEAQAISHEKDEKVLQBEDCEVAENPYTEKNHLDSNRGSSEWAYNG         416       VREFORLSKKCADACEAQAISHEKDEKVLQBEDCEESENPYTEKNHVDSNRGSSEWAYNG         362       VTEFCETCKKCARECESKAITEGERTFEGRSIHNQSGKLQWQNDYNKCLGYWPESG         361       VTEFCETCKKCARECESKAISEGERTFEGRSIHNQSGKLQWQNDHSKCLGYWPESG
Dre-PceA Ddi-DcaA Smu-PceA SL2-PceA <sub>TCE</sub>	476 SPCANCVAVCSWNEVETINHE-WARIATQIPLODAARKFDEWEGYNGPVNPLERLESGY 476 GLCANCVAVCSFNFISTINHE-WARIATRIPLODAARKFDEWFGYSGPVNPLERLESGY 418 GYCGVCVAVCPFTKGNINIHDGVEWLIDNTRFLDPLMLGMDDALGYGAKRNITEVWDGKI 417 GYCGICVAVCPFTKGNINIHDGVEWLIDNTRFLDPLMLGMDDALGYGAKRNITEVWDGKI
Dre-PceA Ddi-DcaA Smu-PceA SL2-PceArce	535 VQN-MVK FWNNPPS IKQ 535 VQN-MVK FWNNPPS IKQ 478 NTYGLDADHFRDIVSFRKDRVKKS 477 NTYGLDADHFRDAVSFRKDRVKKS

**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<sub>TCE</sub> 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<sub>TCE</sub>, respectively.

## References

- 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.
- 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.
- Luijten ML, de Weert J, Smidt H, Boschker HT, de Vos WM, Schraa G, and Stams AJ. 2003. Description of *Sulfurospirillum halorespirans* sp. nov., an anaerobic, tetrachloroethene-respiring bacterium, and transfer of *Dehalospirillum multivorans* to the genus *Sulfurospirillum* as *Sulfurospirillum multivorans* comb. nov. Int J Syst Evol Microbiol 53:787-793.
- Marzorati M, de Ferra F, Van Raemdonck H, Borin S, Allifranchini E, Carpani G, Serbolisca L, Verstraete W, Boon N, and Daffonchio D. 2007. A novel reductive dehalogenase, identified in a contaminated groundwater enrichment culture and in *Desulfitobacterium dichloroeliminans* strain DCA1, is linked to dehalogenation of 1,2-dichloroethane. Appl Environ Microbiol 73:2990-2999.