Interspecies metabolite transfer in a co-culture of *Dehalococcoides* and *Sulfurospirillum* leads to rapid and complete tetrachloroethene dechlorination.

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Abstract

Microbial communities involving dehalogenating bacteria assist in bioremediation of areas contaminated with chlorinated hydrocarbons. To understand molecular interactions between dehalogenating bacteria, we co-cultured two bacterial species dechlorinating chloroethenes: *Sulfurospirillum multivorans*, dechlorinating tetrachloroethene (PCE) to *cis*-1,2-dichloroethene (cDCE) and *Dehalococcoides mccartyi* strains BTF08 or 195, transforming PCE via cDCE to ethene. The interaction of these bacteria cultivated with lactate as electron donor and PCE as electron acceptor was investigated using growth studies, metabolite analysis, microscopy, isotope fractionation and proteomics. Co-cultures exhibited more than 3-fold higher PCE to ethene dechlorination rates than *D. mccartyi* pure cultures. *S. multivorans* provided hydrogen, acetate and the reductive dehalogenase cobamide cofactor to *D. mccartyi*. While *D. mccartyi* 195 dechlorinated cDCE in the presence of norpseudo-B₁₂ produced by *S. multivorans*, cDCE dechlorination by *D. mccartyi* BTF08 depended on the supply of 5,6-dimethylbenzimidazole for producing functional cobamides. Co-cultures were characterized by the formation of aggregates and electron microscopy revealed an extracellular matrix enabling cell-to-cell contact. *D. mccartyi* showed an unusual barrel-like morphology, probably dependent on down-regulation of cell division gene expression, as observed in the co-culture proteome. Only the reductive dehalogenases PteA and VcrA were found in the proteomes of *D. mccartyi* BTF08 during dehalogenation of PCE to ethene.

Introduction

Microbial communities are characterized by numerous interactions involving the exchange and consumption of metabolic products. Molecular hydrogen, for example, is an important electron carrier in syntrophic communities, in which hydrogen is produced by e.g. fermentation and taken up by hydrogen-consuming prokaryotes. This hydrogen consumption leads to a lower hydrogen partial pressure, which allows otherwise thermodynamically unfavorable reactions to proceed. Therefore, the involved bacteria are physiologically dependent on each other (Morris et al 2013, Schink and Stams 2013, Stams and Plugge 2009). The probably carcinogenic groundwater pollutant tetrachloroethene (PCE) is completely dechlorinated to ethene especially in communities involving hydrogen transfer from fermenting bacteria to the obligate hydrogen-consuming *D. mccartyi* (Cichocka et al 2010,
Duhamel and Edwards 2006, Jugder et al 2016, Wei et al 2016). PCE and other organohalides such as hexachlorobenzene or polychlorinated biphenyls are used as terminal electron acceptors by D. mccartyi and further bacteria in an anaerobic respiratory chain coupling the dehalogenation to energy conservation via electron transport phosphorylation (Jugder et al., 2016). This process is termed organohalide respiration (OHR) and involves the corrinoid-containing reductive dehalogenases (RDases) as terminal reductases (Leys et al 2013, Schubert and Diekert 2016, Schubert et al 2018).

However, D. mccartyi is characterized by low growth rates resulting in low dechlorination rates (Löffler et al 2013). Moreover, these bacteria are strictly dependent on specific nutrients and vitamins in their habitats (Zinder 2016). Besides being restricted to hydrogen as electron donor, D. mccartyi uses only acetate plus bicarbonate as carbon source and organohalides as electron acceptors. Additionally, these bacteria are not able to de novo synthesize corrinoids, the obligate cofactor of RDases (Löffler et al 2013, Schipp et al 2013). While proteins for complete corrinoid biosynthesis are usually not encoded in the genomes of D. mccartyi (Türkowsky et al 2018), different studies revealed its ability to salvage and remodel corrinoids, enabling D. mccartyi to restore dechlorination (He et al 2007, Men et al 2014, Yi et al 2012). The functionality of the corrinoid and thus the RDase is directly dependent on the type of the lower base in D. mccartyi. Only three types of corrinoids have so far been described to be functional in D. mccartyi strain 195: 5,6-dimethylbenzimidazolyl-cobamide ([DMB]-Cba), 5-methylbenzimidazolyl-cobamide and 5-methoxybenzimidazolyl-cobamide. Nonfunctional corrinoids e.g. 5-hydroxybenzimidazolyl-cobamide or 7-adeninyl-cobamide ([Ade]Cba) can be converted to functional ones by replacement of the lower ligand in D. mccartyi when 5,6-dimethylbenzimidazole (DMB) is provided (Men et al 2014, Yi et al 2012).

Different studies on dechlorinating communities containing D. mccartyi in association with fermenting, acetogenic and/or methanogenic bacteria revealed higher dechlorination and growth rates than those of pure cultures (DiStefano et al 1992, He et al 2003a, He et al 2003b, Maymo-Gatell et al 1997). It is assumed that cross-feeding and a constant supply of growth factors such as corrinoids and biotin enhance growth (Richardson 2016). Within these communities, non-dechlorinating fermenting bacteria provide hydrogen, acetate and CO₂ from e.g. lactate or butyrate fermentation. The fermenting bacteria are dependent on hydrogen consumers which keep the hydrogen partial pressure low (Cheng et al 2010, Mao et al 2015, Men et al 2012, Richardson et al 2002). For example, co-culture experiments revealed an interspecies hydrogen transfer between Desulfovibrio desulfuricans fermenting lactate
and *D. mccartyi* dechlorinating TCE (He et al. 2007). Acetogens like *Sporomusa ovata* and sulfidogens (*Desulfovibrio*), produce different types of corrinoids which can be used by *D. mccartyi* (Duhamel and Edwards 2007, Guimaraes et al. 1994). An interspecies cobamide transfer was also shown between *Methanosarcina barkeri* strain Fusaro and *D. mccartyi* strain BAV1, GT and FL2, when DMB was present. In the only co-culture of *D. mccartyi* (strains BAV1 and FL2) with a PCE-dechlorinating bacterium, *Geobacter lovleyi*, a corrinoid transfer was also observed (Yan et al. 2012, Yan et al. 2013). In this co-culture, however, hydrogen had to be supplemented. All these studies showed a more robust growth of *D. mccartyi* in co-cultures, resulting in higher dechlorination rates and cell yields than in pure cultures.

One single PCE-dechlorinating bacterium providing hydrogen, acetate and a corrinoid to *D. mccartyi* in a co-culture has never been described. A co-culture with such a syntrophic partner could aid in the optimization of bioremediation and bioaugmentation using *D. mccartyi*-containing cultures. Recently, the PCE to cDCE-respiring Epsilonproteobacterium *Sulfurospirillum multivorans*, capable of *de novo* corrinoid production (Kräutler et al. 2003, Neumann et al. 1996, Schubert 2017), was shown to produce hydrogen and acetate under fermentative growth conditions, e.g. with pyruvate or lactate and without electron acceptor present (Kruse et al. 2017a). In the same study, a co-culture of *S. multivorans* with a methanogen dependent on hydrogen as electron donor was established. Therefore, *S. multivorans* was a promising partner for a co-cultivation with *D. mccartyi*. In addition, it was of interest, whether *D. mccartyi* is able to take up and utilize norpseudo-B_{12} ([Ade]NCba) so far known to be produced exclusively by *S. multivorans* and *S. halorespirans* (Goris et al. 2017, Schubert 2017).

In this study, we investigated the physiological interaction between *D. mccartyi* BTF08 and 195 in co-culture with *S. multivorans*. *S. multivorans* produced hydrogen and acetate during lactate fermentation, which occurred after the electron acceptor PCE was dehalogenated to cDCE. The co-cultures showed an enhanced PCE-to-ethene dechlorination rate compared to the pure cultures. Additionally, interspecies cobamide transfer was detected. Electron microscopic and FISH analysis of co-cultures showed association of both organisms in aggregates. The *S. multivorans/D. mccartyi* co-culture is a candidate for bioaugmentation of PCE-contaminated sites and for studying interactions between different OHRB.
Materials and Methods

Growth conditions of pure cultures

*D. mccartyi* cultures BTF08 and 195 (maintained at UFZ Leipzig) were cultivated in 200 mL serum bottles containing 100 mL bicarbonate-buffered mineral salt medium with 5 mM acetate and 148 nM vitamin B$_{12}$ (cyanocobalamin, ca. 200 µg/L), reduced with Na$_2$S (5% w/w) (Maymó-Gatell et al 1999). Anoxic atmosphere was established by 30 cycles of gassing and degassing with nitrogen and CO$_2$ was added to a final atmosphere of N$_2$:CO$_2$ (75:25 v/v 1.5 bar overpressure). After autoclaving the medium, hydrogen [150 kPa] was applied. PCE (>99% purity, Sigma Aldrich, Steinheim, Germany) and cDCE (97% purity, Sigma Aldrich, Steinheim, Germany) served as electron acceptors and were added with a microliter syringe (Hamilton, Bonaduz, Switzerland) to a final concentration of 0.35 mM (aqueous phase concentration). Re-feeding of the cultures was done with the same amount of PCE or cDCE after complete conversion to ethene. After maximally three re-feeding steps, cultures were transferred [10% (v/v)] into fresh medium. To evaluate the effect of different types and concentrations of B$_{12}$ on dechlorination activities, *D. mccartyi* cultures received 54 nM norpseudo-B$_{12}$ ([Ade]Cba) or 5-OmeBza-B$_{12}$ ([5-OmeBza]Cba), each. Norpseudo-B$_{12}$ was extracted as described previously (Keller et al 2014) from 6 L of *S. multivorans* grown anaerobically with 40 mM pyruvate and 10 mM PCE as described elsewhere (Kruse et al 2017b). 5-OmeBza-B$_{12}$ was obtained from 6L of *Desulfitobacterium hafniense* DCB2 grown anaerobically with 40 mM pyruvate and 10 mM CIOHPA (3-chloro-4-hydroxy-phenylacetate) on a medium amended with 25 µM 5-OmeBza (Schubert et al 2018). *S. multivorans* (DSMZ 12446) was cultured in the same mineral salt medium as *D. mccartyi* with 40 mM lactate and 10mM nominal PCE in hexadecane but without the addition of acetate and hydrogen (Maymó-Gatell et al 1999). All cultivation experiments were performed statically at 28°C in the dark and in biological triplicates.

Growth of co-cultures of *S. multivorans* and *D. mccartyi*

Co-cultures of *S. multivorans* and *D. mccartyi* strain BTF08 or 195 were maintained in the same mineral salt medium as the pure cultures, without acetate and hydrogen. The medium contained 25 mM lactate as the electron donor for *S. multivorans* and 0.35 mM PCE (aqueous-phase concentration) as the electron acceptor. Lactate and PCE was re-fed after depletion in the same concentration as the
initial dose. The B$_{12}$-dependance of the co-cultures was tested with 148 nM vitamin B$_{12}$ serving as the positive control and without vitamin B$_{12}$ amendments. Cultures without vitamin B$_{12}$ received 1 µM DMB (>99% purity, Sigma Aldrich, Steinheim, Germany), where indicated. For isotope fractionation experiments, S. multivorans and Sm/BTF08 co-cultures were cultivated in 50 mL serum bottles with 25 mL medium. Replicate bottles were inoculated at the same time and the dehalogenation process was stopped at different time points by addition of 3 mL 2 M Na$_2$SO$_4$ (pH 1.0).

Quantitative (q)PCR analysis of cell growth

DNA was extracted from 1 mL co-culture taken from different time points during the cultivation experiment using the NucleoSpin Tissue DNA extraction kit according to the manufacturer’s instructions (Macherey-Nagel, Düren, Germany). Quantitative PCR (qPCR) was applied to enumerate Sulfurospirillum and Dehalococcoides 16S rRNA gene copies. The qPCR reaction mixture contained 1 µl of gDNA or DNA standard, 6.25 µl 1x KAPA SYBR Fast master mix (Sigma Aldrich, Steinheim, Germany) and 0.208 µM forward and reverse primer. Primers used were Dhc_sp_16S_fw (5’-GTATCGACCCCTCTCTGTGCCG-3’) and Dhc_sp_16S_rev (5’-GCAAGTTCCTGACTTAACAGGTCGT-3’) for D. mccartyi and Smul_16S_fw (5’-AGGCTAGTTTACTAGAAGGTCGT-3’) and Smul_16S_rev (5’-CAGTCTGATTAGAGTGCTCAG-3’) for S. multivorans. The conditions of the PCR program were as followed: 95°C for 2 min (initial denaturation) followed by 40 cycles of 55°C (S. multivorans primer) or 60°C (D. mccartyi primer) for 20s (annealing), 72°C for 30s (elongation) and 95°C for 10 s (denaturation). Each PCR included a melting curve for verification of specific target DNA amplification. Standard curves were done from extracted gDNA from different cell number preparations of S. multivorans and D. mccartyi strains BTF08 and 195. For this, the cell number preparations contained the same cell number of both organisms from which genomic DNA was isolated. The obtained C$_T$ values were compared with the standard curve to determine the different cell numbers. All samples were conducted in three biological replicates with two corresponding technical replicates or three technical replicates for the calibration curve.

Analytical methods

Ethene and chlorinated ethenes were quantified gas chromatographically with a flame ionization detector (Clarus 500, Perkin Elmer, Rodgau, Germany) and a CP-PoraBOND Q FUSED SILICA 25 m x 0.32 mm column (Agilent Technologies, Böblingen, Germany). A headspace sample from 1 mL
culture as well as from 1 mL gas phase was taken using a gas-tight syringe (Hamilton, Bonaduz, Switzerland) and subsequently analyzed. Ethene and chlorinated ethenes were separated as follows: 4 min at 60°C to 280°C in 10°C min⁻¹ steps. The injector temperature was fixed at 250°C and detector temperature at 300°C. Standard curves of ethene and each chlorinated ethene were recorded for peak area quantification and retention times were compared to known standards. Hydrogen was measured using a thermal conductivity detector (AutoSystem, Perkin Elmer, Rodgau, Germany). Organic acids (e.g. lactate and acetate) were analyzed by HPLC and separated on an AMINEX HPX-87H column (7.8 x 300 mm; BioRad, Munich, Germany) using 5 mM as mobile phase and at a flow rate of 0.7 mL min⁻¹.

**Compound-specific stable isotope analysis**

Determination of the carbon isotope composition of the chlorinated ethenes in pure culture of *S. multivorans* and in co-culture of *S. multivorans* and *D. mccartyi* strain BTF08 was done using gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS; Thermo GC Trace 1320 combined with Thermo-Finnigan MAT 253 IRMS, Bremen, Germany) (Schmidt et al 2014). All samples were analyzed in technical triplicates. 2 mL liquid phase were taken from the respective sample and transferred to a He-flushed 10 mL crimped vial. Of these, 1 mL was taken from the headspace via an autosampler (Thermo TriPlus RSH Autosampler, Bremen, Germany) and injected in a gas chromatograph with a split ratio of 1:5. Chlorinated ethenes were separated on a DB-MTBE column (60 m x 0.32 mm x 1.8 µm, J&W Scientific, Waldbronn, Germany) and the following temperature program: 40°C for 5 min, increase to 250°C by 20°C min⁻¹ and hold for 5 min using helium as carrier gas at a flow rate of 2.0 mL min⁻¹ (Injector at 250°C).

The carbon isotope composition is given in the δ-notation (‰) relative to the Vienna Pee Dee Belemnite standard (Coplen et al 2006). Carbon isotope fractionation was calculated using the Rayleigh equation (eq 1) where R₀ and Rₜ represents the isotope values and C₀ and Cₜ the concentrations at time 0 and t (Elsner et al 2008, Elsner 2010).

\[
\ln \frac{R_t}{R_0} = \epsilon_C \times \ln \frac{C_t}{C_0}
\]  

The carbon isotope enrichment factor (ε₉) relates changes in the concentration of the isotopes to changes in their isotope composition. A two-tailed T-Test was used to calculate the 95 % confidence
interval based on the slope. Standard deviations were obtained from at least triplicate measurements (< 0.5 %).

**B₁₂ extraction and MS analysis**

The B₁₂ content of 100 mL culture was analyzed. For this, the culture volume was reduced to 20 mL using a vacuum concentrator and 0.1 M potassium cyanide was added. After boiling the samples for 20 min, cell debris was removed by centrifugation (10 min, 6700 x g, 8°C). The supernatant was applied onto a C-18 column (CHROMABOND C-18 ec, Macherey-Nagel, Düren, Germany) equilibrated with 5 mL 100% (v/v) methanol and 5 mL ultrapure water (UPW). Washing of the column was done twice with 5 mL UPW and B₁₂-types were eluted with 5 mL 100% (v/v) methanol. The eluate was completely dried in a vacuum dryer and dissolved in UPW prior to analysis. The extract was injected to ultrahigh performance nano-flow liquid chromatography (UHPLC) (Ultimate 3000, Thermo Fisher, Waltham, USA) coupled to mass spectrometer (LC-MS, Orbitrap Fusion, Thermo Fisher, Waltham, USA) via heated electrospray ionization (HESI-II, Thermo Fisher, Waltham, USA). The UHPLC was equipped with a Hypersil Gold C18 column (150 x 2.1 mm, 3 µm film thickness; Thermo Fisher, Waltham, USA) and a C18 guard column (10 x 2.1 mm, Waters, Milford, USA). Chromatographic separation using a gradient method with 0.1% formic acid (A) and methanol (B) as mobile phase was applied as following: 5% B for 1 min, 60 min gradient to 90% B, 4 min at 90% B, 1 min gradient to 5% B and 4 min at 5% B with a constant flow of 0.2 mL min⁻¹ and 25°C column oven temperature. Injection volume was 5 µL. Ionization was set to positive ion mode at 3.5 kV, 35 arbitrary units (Arb) sheath gas, 10 Arb auxiliary gas, 325°C ion transfer tube temperature and 275°C vaporizer temperature. Orbitrap resolution for precursor scan (MS1) was set to 120.000 with a scan range of 300-1600 m/z. Data evaluation was done on the original mass spectra comparing with predicted masses of corrinoids (Supplementary Table S2, for cobalamin standards see Supplementary Figure S3).

**Scanning Electron Microscopy and fluorescence in situ hybridization**

Field emission-scanning electron microscopy (FE-SEM) was performed with co-cultures of *S. multivorans* and *D. mccartyi* strains BTF08 and 195. Cells of 5 mL culture were incubated for 15 min with 2.5% glutaraldehyde and pre-fixed for 2 h on poly-L-lysine coated coverslides (12 mm, Fisher Scientific, Schwerte, Germany). Cover slides were washed three times with 0.1 M sodium cacodylate...
(pH 7.2) (>98% purity, Sigma Aldrich, Steinheim, Germany) and post-fixed for 1 h with 1% osmium
tetroxyde in the same cacodylate buffer. After fixation, samples were dehydrated using different
ethanol concentrations (35%/50%/70%/80%/95%/100% v/v) and incubated for 10 min at each step.
Critical point drying was done in a Leica EM CPD200 Automated Critical Point Dryer (Leica, Wetzlar,
Germany), followed by coating with 6 nm platinum in a BAL-TEC MED 020 Sputter Coating System
(BAL-TEC, Balzers, Liechtenstein). Imaging of the samples was done with a Zeiss-LEO 1530 Gemini
field emission scanning electron microscope (Carl Zeiss, Oberkochen, Germany) at different
magnifications and at 10 kV acceleration voltage. Fluorescence in situ hybridization (FISH) was
performed as described previously (Fazi et al 2008, Matturro et al 2013). In brief, samples were fixed
with formaldehyde (2% v/v final concentration) for 2 h at 4°C and filtered on polycarbonate membrane
filters (47 mm diameter, 0.2 μm pore size, Nucleopore). Filters were stored at -20°C until further
processing. FISH detection of *D. mccartyi* strain BTF08 and 195 was done with Cy3-labeled
DHC1259t and DHC1259c probes and *S. multivorans* detection with FITC-labeled probes SULF
F220ab (Rossetti et al 2008). Imaging of un-aggregated cells was done with an epifluorescence
microscope (Olympus, BX51) combined with an Olympus XM10 camera. Images were analyzed via
Cell-F software. Aggregates were visualized using a confocal laser scanning microscopy (CSLM,
Olympus FV1000).

**Protein extraction and proteome analysis**

Cells were harvested after refeeding of the chloroethene (Supplementary Figure S14). Samples were
processed as described previously (Jehmlich et al 2008). Briefly, protein extraction was performed in
lysis buffer (20 mM HEPES, 1 mM sodium vanadate, 1mM β-glycerolphosphate, 2.5 mM sodium
pyrophosphate, 8 M urea) by three freeze/thaw-cycles and ultrasonic bath treatments. Protein
concentration was determined after protein extraction using the Bio-Rad Bradford reagent (Bio-Rad,
Munich, Germany) and bovine serum albumin as protein standard. Ten μg protein was precipitated
with five-fold ice-cold acetone. Protein pellets were dissolved in 50 μL SDS sample buffer (2% SDS, 2
mM beta-mercaptoethanol, 4% glycerol, 40 mM Tris-HCl pH 6.8, 0.01% bromophenolblue), heated to
90 °C for 5 min and separated on a SDS gel (12.5%). The gel was run until the samples entered the
separating gel. Afterwards, a 3-5 mm protein band from each sample was cut out, destained,
dehydrated, reduced with 10 mM dithiothreitol, alkylated with 100 mM iodoacetamide and
proteolytically cleaved over night at 37°C using trypsin (Promega, Madison, WI, USA). Peptides were
extracted, desalted using C18 ZipTip columns (Merck Millipore, Darmstadt, Germany) and resuspended in 0.1% (v/v) formic acid before LC–MS/MS analysis.

Proteolytic lysates were separated using an Ultimate 3000 RSLCnano liquid chromatographic instrument (Thermo Scientific, Germany). Mass spectrometry was performed on an Orbitrap Fusion mass spectrometer (Thermo Scientific, San Jose, CA, USA) coupled to a TriVersa NanoMate (Advion, Ltd., Harlow, UK). Samples of 5 µL were loaded onto a trapping column (Acclaim PepMap100 C18, 75 µm × 2 cm, Thermo Scientific) using 96% eluent A (0.1% formic acid) and 4% eluent B (0.08% formic acid, 80% acetonitrile) at a flow rate of 5 µL min⁻¹ and separated via a 25 cm analytical column (Acclaim PepMap100 C18, 75 µm × 25 cm, Thermo Scientific) at 35°C using a constant flow rate of 300 nL/min. Peptide separation was achieved by applying a linear gradient of eluent B from 4% to 50% within 100 min. Full MS scans were measured in the Orbitrap mass analyzer within the mass range of 400-1,600 m/z at 120,000 resolution using an automatic gain control (AGC) target of 4x10⁵ and maximum fill time of 60 ms. The MS instrument measured in data-dependent acquisition (DDA) mode using the highest intense ion (top speed, 3 sec cycle time). Positive ion charge states between 2 and 7 were selected for MS/MS. Precursor masses for MS/MS were selected based on highest intensity and excluded from further MS/MS for 30 s to prevent redundancy in MS/MS acquisition. After higher energy collisional (HCD) fragmentation at normalized collision induced energy of 30%, fragment masses were scanned in the Orbitrap mass analyzer at a resolution of 15,000 with 5x10⁴ AGC target and a maximum injection time of 150 ms.

LC-MS/MS data were analyzed using Proteome Discoverer (v2.1, Thermo Scientific). MS/MS spectra were searched against a combination of a *S. multivorans* (3,233 non-redundant protein-coding sequences, downloaded January 2017 from NCBI GenBank, accession number CP007201.1) and a *D. mccartyi* BTF08 database (1,535 non-redundant protein-coding sequences, downloaded December 2016 from NCBI, accession number CP004080.1). A “common repository of adventitious proteins database” (cRAP) was integrated to ensure correct protein identifications. The SEQUEST HT algorithm was used with the following settings: trypsin as cleavage enzyme, oxidation on methionine as dynamic and carbamidomethylation on cysteine as static modification, up to two missed cleavages, precursor mass tolerance set to 10 ppm and fragment mass tolerance to 0.02 Da, respectively. Only peptides with a false discovery rate (FDR) 1%, XCorr ≥2, q-value and the posterior error probability (PEP) ≤0.01 were considered as identified (Supplementary Excel File S1). Quantification of proteins was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.
was performed using the average of the top three peptide MS1 areas. After log10 transformation, the
protein values were normalized to the median of all proteins of a sample, to the median of all proteins
of the respective organism in that sample and to the median of all samples and scaled so that the
global minimum is zero. Two outliers of the co-culture proteome replicates with only 11 (C3) and 92
(P1, see Supplementary Excel File S1) protein identifications, compared to at least 317 protein
identifications from the other D. mccartyi pure cultures, were excluded from the analysis. Proteins with
only one out of three possible quantitative value per sample counted as identified only. P-values were
calculated using a two-tailed, homoscedastic student’s t-test and multiple corrected with the
Benjamini-Hochberg method. Figures were created using an in-house written R-script with the
packages gplots, ggplot2, ggbioplot, dplyr, miscTools and vegan. The non-parametrical multiple
dimensional scaling (nMDS)-analysis was performed with the anosim-function of the vegan package in
R (v3.4.1). *Pairwise Indicator Species Analysis* was used to identify proteins that were significantly
associated with the different cultivation conditions (Kanehisa et al 2016, Marc and Pierre 1997). Indval
scores and significances were calculated according to Malik et al. (Malik et al 2018).
Results

Growth and dechlorination in *Sulfurospirillum (Sm)/Dehalococcoides (Dhc)* co-cultures

To ensure optimal cultivation conditions for both organisms in the co-culture, *S. multivorans* was cultivated with lactate and PCE in *D. mccartyi* medium. We observed an equal growth behavior of *S. multivorans* compared to that in the medium routinely used for cultivation (Scholz-Muramatsu et al 1995), data not shown). With lactate as electron donor and PCE as electron acceptor, a co-culture of *S. multivorans* and *D. mccartyi* BTF08 maintained over 10 transfers under the same conditions dechlorinated PCE to stoichiometric amounts of ethene within eight days after inoculation (Figure 1A, Table 1). The culture was re-fed with PCE at day 8 and day 12 after inoculation. After re-feeding, the dechlorination rate increased 2.8-fold (Figure 1A, Table 1) and complete dechlorination of PCE occurred within three days. PCE was dechlorinated to cDCE within two days. To check whether *S. multivorans*, known for its high dehalogenation rate, is responsible for this fast dechlorination, the stable carbon isotope fractionation patterns of pure and co-cultures were compared. No significant differences in the fractionation of PCE between *S. multivorans* pure and the Sm/Dhc co-cultures were found. The isotope fractionation pattern of PCE in the co-culture did not change significantly, and ranged from -30.2 ± 0.09 to -29.5 ± 0.12 ‰ at 70% of transformed PCE (Supplementary Figure S1A). A low but significant fractionation was measured in the pure culture of *S. multivorans* (from -29.2 ± 0.05 to -27.6 ± 0.11 ‰ at 56% of transformed PCE, Supplementary Figure S1B). The isotope enrichment factors were calculated using the Rayleigh equation and were in the same range while differing largely from that of a *D. mccartyi* BTF08 pure culture (Sm/BTF08 co-culture: $\epsilon_C = -0.4 \pm 0.3$ ‰; *S. multivorans* pure culture: $\epsilon_C = -2.0 \pm 0.4$ ‰, *D. mccartyi* BTF08: $\epsilon_C = -5.0$ to -9.0 ‰ (Franke et al., unpublished)). The fast dechlorination of PCE to cDCE was also reflected in a fast increase of the *S. multivorans* cell number, corresponding to approximately 2.5 cell doublings during the first two days until PCE was completely dechlorinated to cDCE (Figure 1C). After this respiratory growth, lactate fermentation of *S. multivorans* started with a weaker growth observed. *D. mccartyi* BTF08 needed seven days for one cell doubling, but growth was also observed during the first two days. For both organisms, a correlation between dechlorination and growth was observed. The ratio between *S. multivorans* and *D. mccartyi* BTF08 cells changed from initially 2.6:1 to 5.9:1 after 2 days and 4.6:1 after 15 days and two re-feeding steps. After 12 days, when the second dose of PCE was completely
dechlorinated to ethene, lactate was completely consumed, therefore lactate and PCE were re-fed. Acetate production occurred continuously during the whole dechlorination process (Figure 1E). No hydrogen was detected in the gas phase at any point (data not shown). The dechlorination rate (PCE to ethene) of the Sm/BTF08 co-culture was 4.5-fold faster compared to the *D. mccartyi* BTF08 culture. In the latter, PCE was completely reduced to ethene within 35 days at a rate of 0.9 ± 0.03 µmol/bottle/day, which increased after re-feeding to 1.4 µmol/bottle/day (Table 1, Supplementary Figure S2A).

Similar growth characteristics and dechlorination behavior was observed in the Sm/195 co-culture cultivated under the same conditions, except that vinyl chloride (VC) was the major dechlorination product. The first dose of PCE was dechlorinated within 7 days stoichiometrically to VC (Figure 1B). The increase of cell number was slightly lower for *S. multivorans* and slightly higher for *D. mccartyi* 195 compared to the Sm/BTF08 co-culture (Figure 1D). After the second dose of PCE was dechlorinated to VC, a low amount of ethene was produced starting on day 10, reaching 10.4 µmol ethene bottle\(^{-1}\) after day 15. The Sm/195 co-culture reduced PCE to VC more than 3-fold faster than the *D. mccartyi* 195 pure culture. After re-feeding of PCE, this rate increased to nearly 8-fold faster (Table 1, Supplementary Figure S2B).

### 2. Corrinoid transfer in co-cultures and the effect of the lower corrinoid ligand

*D. mccartyi* strains rely on externally provided corrinoids for dehalogenation and growth, which was also tested for *D. mccartyi* strains BTF08 and 195, where only negligible cDCE dechlorination after 100 days was observed without addition of corrinoid (Figure 2A and D). Therefore, it was of interest whether *S. multivorans* is able to provide functional corrinoids for *D. mccartyi* strains BTF08 and 195. In Sm/BTF08 co-cultures without the amendment of vitamin B\(_{12}\), stoichiometric dechlorination of PCE to cDCE was obtained (~30 µmol/bottle/day) (Figure 2B). No further dechlorination of cDCE to VC or ethene was detected, indicating that *S. multivorans* alone was responsible for the dechlorination. When cDCE dechlorination stalled in the Sm/BTF08 co-culture, 1 µM 5,6-dimethylbenzimidazole (DMB) was added, which resulted in subsequent cDCE dechlorination (Figure 2C). Ethene production rates from cDCE (3.6 ± 0.2 µmol/bottle/day) were similar to the co-culture amended with vitamin B\(_{12}\) (4.1 ± 0.2 µmol/bottle/day). The dechlorination rate increased after re-feeding with PCE to 8
µmol/bottle/day. In this set-up, three different types of corrinoids were found by mass spectrometric analysis: [Ade]NCba, [DMB]NCba and [DMB]Cba (Supplementary Figure S4).

Interestingly and in contrast to the Sm/BTF08 co-culture, the Sm/195 co-culture without vitamin B₁₂ amendment (-B₁₂) dechlorinated PCE to VC, although at low rates (Figure 2E). After 35 days, PCE was re-fed, and cDCE to VC dechlorination increased to a 4-fold higher dechlorination rate (3.6 µmol/bottle/day). The only corrinoid detected in the Sm/195 co-culture -B₁₂ was [Ade]NCba (Supplementary Figure S5). No significant increase in cDCE dechlorination was observed when 1 µM DMB was added to the Sm/195 co-culture (Figure 2F). Like in the Sm/BTF08 co-culture [Ade]NCba, [DMB]NCba and [DMB]Cba was found by mass spectrometric analysis when DMB was added (Supplementary Figure S6). To confirm whether D. mccartyi 195 can use [Ade]NCba for dechlorination of cDCE to VC, we amended a pure culture with [Ade]NCba isolated from S. multivorans (Supplementary Figure S7A). Dechlorination of 50 µmol cDCE to VC was detected in 22 days. D. mccartyi 195 pure cultures amended with [5-OMeBza]Cba showed slightly faster dechlorination and the cDCE was converted within 16 days into VC (Supplementary Figure S7B). Only the amended corrinoid types were detected in the cultures via MS, impurities and rearrangement of corrinoids could be therefore excluded (Supplementary Information Figure S8 and S9).

3. Electron microscopy and FISH analysis of formed cell aggregates

After about 25 transfers on lactate and PCE, all co-cultures formed spherical aggregates up to 2 mm in diameter (Figure 3A). Field emission-scanning electron microscopy (FE-SEM) was applied to uncover the cell morphology and cell distribution in these aggregates. After preparation for FE-SEM, the sizes of the aggregates were lower (30 to 200 µm, Figure 3B and C.). Electron micrographs of both co-cultures revealed a compact network of S. multivorans and D. mccartyi cells coiled around net-forming filament-like structures. The cells were embedded in an extracellular matrix (ECM) which might aid cell-to-cell contact (Figure 3D, Supplementary Figure S10). S. multivorans could be distinguished from D. mccartyi by FISH with specific oligonucleotide probes targeting 16S rRNA and 3-dimensional imaging showed a spatial organization and an almost equal distribution of both species within the aggregates (Figure 3E, Supplementary Figure S12). The high resolution of the confocal laser scanning microscopy enabled visualization of single cells and revealed the same morphologies as in the
electron micrographs. In the FISH pictures, the sizes of the aggregates ranged from 50 to 100 µm in diameter.

*S. multivorans* revealed a typical helical rod shaped cell structure and a size of 2 to 5 µm to 0.5 µm at magnifications of around 10,000x and 20,000x in FE-SEM as described previously (Scholz-Muramatsu et al 1995) (Figure 4, Supplementary Figure S10). However, the typical polar flagellum was only observed in a few cells and several flagella seemed to be detached from cells, possibly a part of the ECM (Figure 4, Supplementary Figure S10). *D. mccartyi* showed atypical cell morphology in the co-cultures. Microscopic analysis of the pure culture revealed a disc-shaped irregular coccus of 0.5 µm diameter (Figure 4a, Supplementary Figure S11), as previously described (Löffler et al 2013), whereas the *D. mccartyi* strains in the co-culture showed 0.5 µm large barrel-like cells with a flattened cell pole at one side and a ring-shaped septum (Figure 4, Supplementary Figure S10). FISH analysis of isolated cells in the co-culture identified unequivocally both organisms and confirmed the presence and unusual morphology of *D. mccartyi* (Supplementary Figure S12 and S13).

**4. Proteomics of pure and co-cultures**

We applied a label-free shotgun proteomics approach to identify changes in protein abundance during PCE dechlorination in pure and co-cultures (with and without vitamin B12) with *D. mccartyi* BTF08 and *S. multivorans*. Samples for proteomic analyses were taken after one re-feeding with PCE or cDCE (pure *D. mccartyi* cultures) or 12 hours after the third three re-feeding with PCE (co-cultures). While PCE, TCE and cDCE were the dominant chlorinated ethenes in the co-cultures, cDCE and VC were the dominant chlorinated ethenes in the pure culture when harvested (Supplementary Figure S14). The clustering approach nMDS based on quantified proteins revealed a significant separation between pure and co-cultures (Dhc BTF08 proteins p=0.001, Sm proteins p=0.004), indicating a difference of the physiology of both organisms depending on the cultivation and (Supplementary Figure S15). A multi-level pattern analysis was applied to determine indicator proteins for a given condition (i.e. co-culture vs. pure culture) to better understand the functional changes occurring during the dehalogenation in the co-culture. This method was more promising than calculating the changes in
protein abundances between two conditions, since it was possible to compare all five conditions in the
protein indicator analysis (Supplementary Excel File S2).

We detected only two RDase proteins of BTF08 among all proteomes, the gene products of btf_1393
and btf_1407 (VcrA). The two reductive dehalogenases PceA and TceA, putatively involved in
dehlorination of PCE to cDCE, were not identified under any condition. While VcrA was one of the
most abundant proteins under all tested conditions (Figure 5, Supplementary Excel File S1), the gene
product of btf_1393 was more abundant in the two co-cultures (Figure 5, Supplementary Excel File
S2). A BLASTp search against the NCBI nr database revealed that the btf_1393 amino acid sequence
was almost identical (99% or 497/498 amino acid sequence identity over the whole length) to an RdhA
from D. mccartyi 11a5, encoded by 11a5_1355 and characterized as a novel PCE reductive
dehalogenase PteA (Zhao et al 2017). In S. multivorans, the proteins encoded in the organohalide
respiratory gene region including the PCE reductive dehalogenase PceA were present among all
conditions as described previously (Goris et al 2015), albeit more prominent in the pure culture
(Supplementary Excel File S1).

Only a few proteins encoded in the genomes of S. multivorans or D. mccartyi are annotated to play a
potential role in the formation of an ECM, such as pili or proteins involved in the formation of
extrapolymeric substances. The proteins which are part of a putative type II pilus system in D. mccartyi
(encoded by btf_1229 to btf_1240) were present in low to medium amounts among all conditions
(about median [Med] to lower than Med minus standard deviation [SD]) but not significantly more
abundant in the co-cultures. Many proteins involved in flagellar motility of S. multivorans were lower
abundant in the co-culture, with most of the Flg and Fli proteins not detected (Supplementary Excel
File S1).

Protein indicator analysis revealed further, that several outer membrane porins of S. multivorans,
(encoded by SMUL_0494, SMUL0693, SMUL_0926 and SMUL_2351), were significant indicator
proteins for the co-cultures. The main porins of S. multivorans (SMUL_0627, SMUL_0636 and
SMUL_3013) detected under all conditions and in the previous proteomic studies (Gadkari et al 2018,
Goris et al 2015) were not significantly altered. Interestingly, several molybdopterin oxidoreductases of
S. multivorans were highly abundant only in the co-culture (Supplementary Table S3). Among these
enzymes often involved in anaerobic catabolism, were e.g. a nitrate reductase (SMUL934-939) and
two polysulfide reductases (SMUL0342-343 and SMUL3274-3275).
Proteins related to cell division (tubulin/GTPase encoded by btf_0551, FtsZ by btf_0595, FtsH encoded by btf_357, a cell division trigger factor encoded by btf_0631) were more abundant or exclusively quantified in pure cultures (Figure 5, Supplementary Table S4, Supplementary Excel File S2). A hypothetical protein encoded close to the tubulin (btf_0548) was also highly abundant (>Med+SD or >Med) only in cells of the pure culture, while it could not be quantified in any of the co-cultures.

The amidohydrolase CbiZ (btf_610), responsible for cleavage of the nucleotide loop of corrinoids, was quantified only in both co-culture conditions in medium abundance (Figure 5). Several other proteins involved in corrinoid salvage could be quantified in all cultures, except the nicotinate-nucleotide-dimethylbenzimidazole phosphoribosyltransferase CobT (btf_0613), which was not found in the co-culture amended with DMB. The L-threonine 3-O-phosphate decarboxylase CobD, which synthesizes the linke of the lower base and the corrinoid ring of cobamides, could not be quantified in any culture.

Subunits of the vitamin B\textsubscript{12} transporter were detected in all cultures not differing significantly in abundance.

Discussion

In this study, we investigated the dechlorination of PCE to ethene (or vinyl chloride) in co-cultures of \textit{S. multivorans} with \textit{Dehalococcoides mccartyi} BTF08 and 195. Dechlorination profiles, metabolite analysis and growth studies point to a biphasic physiology of the co-culture. In the first stage, when PCE or TCE serve as electron acceptors, \textit{S. multivorans} grows by organohalide respiration with lactate as electron donor. In this phase, \textit{D. mccartyi} does not contribute significantly to PCE dechlorination as indicated by the stable isotope fractionation pattern and the corresponding enrichment factors. This can be explained by the insufficient supply of \textit{D. mccartyi} with hydrogen as electron donor due to the lack of (or very low) hydrogen production by \textit{S. multivorans} during respiratory growth. In the second phase of the co-culture, when PCE has been completely converted to cDCE after less than two days, \textit{S. multivorans} grows via fermentation of lactate mainly to acetate, CO\textsubscript{2} and hydrogen. It has been shown earlier that, for thermodynamic reasons, lactate can only be utilized fermentatively by \textit{S. multivorans} when the hydrogen concentration is very low (Kruse et al. 2018).
These conditions are fulfilled in the co-culture by *D. mccartyi* utilizing H\(_2\) as electron donor for reductive dehalogenation of cDCE or VC in this phase (Figure 6). As expected, PCE was completely dechlorinated to ethene in the Sm/BTF08 co-culture and mainly to VC with minor amounts of ethene in Sm/195. A similar slow and incomplete VC dechlorination was found for pure cultures of the latter strain (Maymó-Gatell et al 2001) and co-cultures of *D. mccartyi* 195 with either *Desulfovibrio vulgaris* Hildenborough or *Syntrophomonas wolfei*, (Mao et al 2015, Men et al 2012). cDCE is presumably converted to ethene by VcrA of BTF08. This is supported by the proteomic analysis described here, in which VcrA of *D. mccartyi* BTF08 is highly abundant and the predominant RDase in all cultures. A highly similar VcrA ortholog of *D. mccartyi* strain VS (99% amino acid sequence identity) was biochemically characterized and described to dechlorinate cDCE and VC (Parthasarathy et al 2015).

Interestingly, neither TceA, nor PceA, which were suggested to be responsible for dechlorination of PCE to VC (Pöritz et al., 2013), were detected in any of the samples. The only other RDase, encoded by btf_1393, was detected mainly in the co-culture. An identical ortholog of this RDase was recently characterized as PteA, a novel PCE reductive dehalogenase dechlorinating PCE to TCE in *D. mccartyi* strain 11a5 (Zhao et al 2017). *D. mccartyi* 195 was not subject to proteomic investigation in our study. However, previous studies suggested that TceA of this organism is involved in cDCE to VC dechlorination and that VC is further dechlorinated slowly by the same enzyme (Magnuson et al 2000, Maymó-Gatell et al 2001).

The Sm/BTF08 co-culture was not capable of cDCE dechlorination to ethene without amendment of vitamin B\(_{12}\), indicating that *D. mccartyi* BTF08 cannot use the norpseudo-B\(_{12}\) ([Ade]NCba) synthesized by *S. multivorans* for cDCE dechlorination. Complete dechlorination to ethene by *D. mccartyi* BTF08 was restored by the addition of the lower ligand DMB, indicating that the non-functional adenosyl ligand could be replaced by DMB. This assumption is supported by a previous study, in which *D. mccartyi* was shown to incorporate different lower ligands into cobalamins (Yi et al., 2012). Therefore, it was not surprising that DMB-containing cobalamins were detected in the Sm/Dhc co-cultures. In addition to [Ade]NCba, which is produced by *S. multivorans*, [DMB]NCba and [DMB]Cba were found. Most probably, [DMB]NCba was synthesized by *S. multivorans* after addition of DMB besides [Ade]NCba, since the organism is able to incorporate different benzimidazoles and generate the corresponding cobalamins (Keller et al 2018, Schubert 2017). [DMB]Cba could then be produced during salvaging and remodeling of available [Ade]NCba and/or [DMB]NCba by *D. mccartyi*. It can,
however, not be excluded from our data that [DMB]NCba can be used as well by *D. mccartyi* BTF08 for cDCE or VC dechlorination. The nucleotide loop cleavage is most likely mediated by the adenosylcobinamide hydrolase CbiZ, as shown for CbiZ of *Rhodobacter sphaeroides* (Gray and Escalante-Semerena 2009a, Gray and Escalante-Semerena 2009b). Of the two CbiZ encoded in the genome of *D. mccartyi* BTF08, one was detected in the proteome and showed a higher abundance in the co-culture compared to the pure culture. These results present evidence of the involvement of CbiZ in exchanging the complete nucleotide loop in *D. mccartyi*.

Opposed to the cDCE accumulation in Sm/BTF08 co-cultures grown without DMB, the Sm/195 co-culture dechlorinated PCE to VC in media without vitamin B$_{12}$ or DMB. This indicates cDCE to VC conversion by strain 195 with [Ade]NCba produced by *S. multivorans*. However, previous studies showed [Ade]Cba (or other cobalamins with adenine as lower ligand) to be non-functional in *D. mccartyi* strain 195 with TCE as electron acceptor (Yi et al 2012). Since RDase gene expression is often dependent on the electron acceptor present (Türkowsky et al 2018), cDCE in the Sm/195 co-culture might have induced a yet unknown RDase dechlorinating cDCE to VC with [Ade]NCba as cofactor. Alternatively, TceA containing [Ade]NCba could catalyze cDCE to VC dechlorination, but not TCE dechlorination to cDCE. The mechanism of cobalamin transfer from *S. multivorans* to *D. mccartyi* is not yet known. Most likely, cobalamin is released upon cell lysis or degradation of periplasmic PceA and possibly excreted through porins of *S. multivorans*, some of which were higher abundant in co-cultures.

The cultivation of *D. mccartyi* in association with *S. multivorans* led to the formation of cell aggregates, which is a common characteristic of obligate syntrophic interactions often found in e.g. acetogenic and methanogenic communities (Hulshoff Pol et al 2004, Ishii et al 2005, Stams et al 2012). FISH staining confirmed the close association of both organisms and provided first insights into the organization and spatial distribution of the organisms within aggregates in a dechlorinating mixed culture. Electron microscopic analysis of the co-cultures revealed cells in close physical contact embedded in an ECM consisting of extracellular polymer substances (EPS)-like structures and surrounding filaments including also flagella detached from *S. multivorans* cells. The decrease of intermicrobial distances and establishment of cell-to-cell contacts should lead to increased metabolite fluxes (e.g. hydrogen) between species according to Fick's law (Schink and Thauer 1988), ultimately enhancing growth and dechlorination rates. Experimentally, this was shown, for example, during syntrophic propionate
conversion of *Pelotomaculum thermopropionicum* SI and *Methanothermobacter thermoautotrophicus* ΔH where interspecies hydrogen transfer was calculated to be optimal in aggregates (Ishii et al 2006).

EPS-like substances and flagella most likely contribute to a stabilization of aggregates by adhesion and attachment of the cells (Grotenhuis et al 1991, Ishii et al 2005). In association with *S. multivorans*, *D. mccartyi* showed a barrel-like morphology with a septum-like structure, which is in contrast to the irregular disc-shaped coccus as found in pure culture. This morphology might be caused by the down-regulation of proteins involved in cell division in the co-culture. One of these proteins, FtsZ, localized at the cell division site, was shown to play a key role in cytokinesis in *E. coli*. It was shown to be responsible for septal invagination of the cell wall and cytoplasmic membrane by forming a ring-shaped septum followed by cell division (Bi and Lutkenhaus 1991, de Boer et al 1992, Lutkenhaus 1993). Its down-regulation might hamper a complete membrane constriction resulting in slower cell division that might cause the observed barrel-like cell morphologies. However, this conclusion is speculative and needs more investigation. Since electron micrographs of co-cultures with *D. mccartyi* are scarce, it is not possible to state whether the unusual cell morphology is specific for the partnership with *S. multivorans* or whether it is found frequently for co-cultures containing *D. mccartyi*.

With *S. wolfei* as syntrophic partner, disc-like cell structures typical for *D. mccartyi* strain 195 were observed (Mao et al 2015).

This study provides first insights into the interactions of *S. multivorans* in association with other organohalide-respiring bacteria. Dechlorinating microbial communities sometimes reveal the presence of *Sulfurospirillum* spp., but the functional role of these Epsilonproteobacteria is unexplored (Goris and Diekert 2016). We observed that interspecies hydrogen and cobamide transfer in the co-culture resulted in fast and complete dechlorination of PCE to ethene. *S. multivorans* could provide all growth factors required by *D. mccartyi*, including hydrogen and cDCE as energy source, acetate as carbon source and cobamides as RDase cofactors (Figure 6). It is the first study in which *D. mccartyi* was provided with all nutrients required for growth by its syntrophic partner. Additionally, PCE to cDCE dechlorination is sped up by the fast dechlorination rate of *S. multivorans*. This is of high interest for bioremediation attempts using *Dehalococcoides*-containing mixed cultures, since electron donor and cobalamin limitations often impede *Dehalococcoides* dechlorination activities. Thus, the established co-culture efficiently detoxifying PCE to ethene has potential for bioaugmentation processes.
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Conflict of interest

The authors declare no conflict of interests.

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**Table 1: Dechlorination rates** of different co-culture set-ups of *S. multivorans* and *D. mccartyi* BTF08 or 195 to the corresponding end product VC or ethene. n. a. - not applied.

| Organism(s) | Electron donor | 1st dose PCE | 2nd dose PCE | 3rd dose PCE |
|-------------|----------------|--------------|--------------|--------------|
| **With vitamin B<sub>12</sub>** |
| *D. mccartyi* BTF08 | H<sub>2</sub> | 0.9 ± 0.03 | 1.4 ± 0.09 | n.a. |
| *D. mccartyi* 195 | H<sub>2</sub> | 1.5 ± 0.04 | 3.3 ± 0.17 | n.a. |
| Sm/BTF08 | Lactate | 4.1 ± 0.2 | 11.6<sup>a</sup> | 11.6<sup>a</sup> |
| Sm/195 | Lactate | 4.8 ± 0.1 | 11.6<sup>a</sup> | 11.6<sup>a</sup> |
| **Without vitamin B<sub>12</sub>** |
| Sm/BTF08 | Lactate | - | - | - |
| Sm/195 | Lactate | 0.9 ± 0.004 | 3.6<sup>a</sup> | 3.6<sup>a</sup> |
| **Without vitamin B<sub>12</sub> + DMB** |
| Sm/BTF08 | Lactate | 3.6 ± 0.2 | 8.0<sup>a</sup> | 8.0<sup>a</sup> |
| Sm/195 | Lactate | 0.8 ± 0.05 | 4.3<sup>a</sup> | 4.3<sup>a</sup> |

<sup>a</sup>. no standard deviation, since the PCE values for re-feeding are theoretical values of the amount PCE added to the culture and were not measured.
Figure 1: Dechlorination of chlorinated ethenes, growth and metabolite analysis of *S. multivorans/D. mccartyi* co-cultures with vitamin B₁₂ amendment. (A) PCE dechlorination of Sm/BTF08 and (B) Sm/195. (C) Growth curve of Sm/BTF08 and (D) Sm/195. (E) Lactate consumption and acetate production of Sm/BTF08 and (F) Sm/195. Arrows indicate the time points of re-feeding the culture with PCE (A-D) or lactate (E and F). Broken red lines with open symbols represent theoretical, not analytical, values of the PCE concentration as added to the culture. Please note the secondary y-axes for *D. mccartyi* cell numbers in C and D. Negative controls were run with autoclaved cells (abiotic controls, C). Standard deviation of three independent biological replicates (N=3) is represented by error bars (not visible when smaller than the used symbol). Σ - mass balance; sum of PCE, TCE, cis-DCE, VC and ethene.

Figure 2: Dechlorination of *S. multivorans/D. mccartyi* co-cultures without addition of vitamin B₁₂. (A) Strain BTF08 with cDCE as electron acceptor (negative control). (B) Sm/BTF08 with PCE as the electron acceptor. (C) Sm/BTF08 with PCE as electron acceptor and amendment of 1 µM DMB. (D) Strain 195 with cDCE as electron acceptor (negative control). (E) Sm/195 with PCE as the electron acceptor. (F) Sm/195 with PCE as electron acceptor and amendment of 1 µM DMB. Please note the different time scales. All growth experiments were conducted in biological triplicates (N=3). Arrows indicate re-feeding of PCE. Σ - mass balance; sum of PCE, TCE, cDCE, VC and ethene.

Figure 3: Microscopic analysis of cell aggregates in co-cultures of *S. multivorans* and *D. mccartyi* strain 195 and BTF08. (A) Serum bottle (200 mL) of a Sm/BTF08 co-culture showing cell aggregates. (B) Light microscopic image of a Sm/BTF08 aggregate. (C, D) Scanning electron micrographs of an aggregate of Sm/195. (E) Confocal laser scanning image of FISH stained aggregates of Sm/BTF08. Red - *S. multivorans*, green - *D. mccartyi*.

Figure 4: Different cell morphologies of *D. mccartyi* strain BTF08 cells. In pure culture (A) and co-culture with *S. multivorans* (B, C). White arrows indicate flagella and yellow arrows indicate ring-shaped septum.

Figure 5: Protein abundances of reductive dehalogenases and significant indicator proteins of Dhc BTF08 in pure and co-cultures. Average protein abundance values under different cultivation conditions are shown. Abundances represent log₁₀ fold changes of the median, scaled to zero. The median of all proteins is at about 2.3 (see Supplementary Figure S16, which also shows each replicate in a scatter plot graph). Four replicates (N=4) were used for proteomic analyses; one of the four replicates were discarded from each of the *D. mccartyi* pure cultures (see Methods). Error bars represent standard deviation which is covered completely by the symbol if <0.1. D - *D. mccartyi* BTF08 pure culture, C - Smul/BTF08 co-culture, CbiZ - adenosylcobinamide amidohydrolase (btf_610), FtsZ - cell division protein (btf_0595), FtsZ/Tubulin GTPase (btf_0551), VcrA - vinyl chloride reductive dehalogenase (btf_1407), btf_1393 - reductive dehalogenase homolog to 11a5_1355 of *D. mccartyi* 11a5.

Figure 6: Interspecies metabolite transfer of *Sulfurospirillum multivorans* and *Dehalococcoides mccartyi*. PCE is dechlorinated to cDCE by *S. multivorans* with electrons from lactate oxidation. After depletion of PCE, *S. multivorans* switches to fermentative metabolism, thereby generating hydrogen which is consumed by *D. mccartyi* as electron donor. The electron acceptor for *D. mccartyi* is cDCE and is further dechlorinated to ethene or VC by *D. mccartyi*. *S. multivorans* synthesizes norpseudo B₁₂ ([Ade]NCba, ●) and [DMB]NCba, ● when DMB is amended. Both can be salvaged and remodeled by *D. mccartyi* into [DMB]Cba (● with a y-shaped linker). The interspecies cobamide transfer is indicated by yellow arrows. A discrimination between norcobamide or cobamide incorporation into the *D. mccartyi* RDases is not depicted here, since the data do not allow a conclusion on the cobalamin nucleotide loop type.
