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 (*c*DCE) and *Dehaloccoides mccartyi* strains BTF08 or 195, transforming PCE via *c*DCE 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 *c*DCE in the presence of norpseudo-B_{12} 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 CO_2 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 *c*DCE-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 *c*DCE. 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_2S (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 *c*DCE (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 *c*DCE 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 ClOHPA (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_2SO_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'-GTATCGACCCTCTCTGTGCCG-3') and Dhc_sp_16S_rev (5'-GCAAGTTCCTGACTTAACAGGTCGT-3') for *D. mccartyi* and Smul_16S_fw (5'-AGGCTAGTTTACTAGAACTTAGAG-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

165 culture as well as from 1 mL gas phase was taken using a gas-tight syringe (Hamilton, Bonaduz,
166 Switzerland) and subsequently analyzed. Ethene and chlorinated ethenes were separated as follows:
167 4 min at 60°C to 280°C in 10°C min^{-1} steps. The injector temperature was fixed at 250°C and detector
168 temperature at 300°C. Standard curves of ethene and each chlorinated ethene were recorded for peak
169 area quantification and retention times were compared to known standards. Hydrogen was measured
170 using a thermal conductivity detector (AutoSystem, Perkin Elmer, Rodgau, Germany). Organic acids
171 (e.g. lactate and acetate) were analyzed by HPLC and separated on an AMINEX HPX-87H column
172 (7.8 x 300 mm; BioRad, Munich, Germany) using 5 mM as mobile phase and at a flow rate of 0.7 mL
173 min^{-1}.

174 **Compound-specific stable isotope analysis**

175 Determination of the carbon isotope composition of the chlorinated ethenes in pure culture of *S.*
176 *multivorans* and in co-culture of *S. multivorans* and *D. mccartyi* strain BTF08 was done using gas
177 chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS; Thermo GC Trace 1320
178 combined with Thermo-Finnigan MAT 253 IRMS, Bremen, Germany) (Schmidt et al 2014). All samples
179 were analyzed in technical triplicates. 2 mL liquid phase were taken from the respective sample and
180 transferred to a He-flushed 10 mL crimped vial. Of these, 1 mL was taken from the headspace via an
181 autosampler (Thermo TriPlus RSH Autosampler, Bremen, Germany) and injected in a gas
182 chromatograph with a split ratio of 1:5. Chlorinated ethenes were separated on a DB-MTBE column
183 (60 m x 0.32 mm x 1.8 µm, J&W Scientific, Waldbronn, Germany) and the following temperature
184 program: 40°C for 5 min, increase to 250°C by 20°C min^{-1} and hold for 5 min using helium as carrier
185 gas at a flow rate of 2.0 mL min^{-1} (Injector at 250°C).

186 The carbon isotope composition is given in the δ-notation (‰) relative to the Vienna Pee Dee
187 Belemnite standard (Coplen et al 2006). Carbon isotope fractionation was calculated using the
188 Rayleigh equation (eq 1) where R_0 and R_t represents the isotope values and C_0 and C_t the
189 concentrations at time 0 and t (Elsner et al 2008, Elsner 2010).

190 $$ln\frac{Rt}{R0} = \varepsilon C * ln\frac{Ct}{C0} \qquad (1)$$

191 The carbon isotope enrichment factor (ε_C) relates changes in the concentration of the isotopes to
192 changes in their isotope composition. A two-tailed T-Test was used to calculate the 95 % confidence

193 interval based on the slope. Standard deviations were obtained from at least triplicate measurements
194 (< 0.5 ‰).

195 **B_{12} extraction and MS analysis**

196 The B_{12} content of 100 mL culture was analyzed. For this, the culture volume was reduced to 20 mL
197 using a vacuum concentrator and 0.1 M potassium cyanide was added. After boiling the samples for
198 20 min, cell debris was removed by centrifugation (10 min, 6700 x g, 8°C). The supernatant was
199 applied onto a C-18 column (CHROMABOND C-18 ec, Macherey-Nagel, Düren, Germany)
200 equilibrated with 5 mL 100% (v/v) methanol and 5 mL ultrapure water (UPW). Washing of the column
201 was done twice with 5 mL UPW and B_{12}-types were eluted with 5 mL 100% (v/v) methanol. The eluate
202 was completely dried in a vacuum dryer and dissolved in UPW prior to analysis. The extract was
203 injected to ultrahigh performance nano-flow liquid chromatography (UHPLC) (Ultimate 3000, Thermo
204 Fisher, Waltham, USA) coupled to mass spectrometer (LC-MS, Orbitrap Fusion, Thermo Fisher,
205 Waltham, USA) via heated electrospray ionization (HESI-II, Thermo Fisher, Waltham, USA). The
206 UHPLC was equipped with a Hypersil Gold C18 column (150 x 2.1 mm, 3 µm film thickness; Thermo
207 Fisher, Waltham, USA) and a C18 guard column (10 x 2.1 mm, Waters, Milford, USA).
208 Chromatographic separation using a gradient method with 0.1% formic acid (A) and methanol (B) as
209 mobile phase was applied as following: 5% B for 1 min, 60 min gradient to 90% B, 4 min at 90% B, 1
210 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
211 temperature. Injection volume was 5 µL. Ionization was set to positive ion mode at 3.5 kV, 35 arbitrary
212 units (Arb) sheath gas, 10 Arb auxillary gas, 325°C ion transfer tube temperature and 275°C vaporizer
213 temperature. Orbitrap resolution for precursor scan (MS1) was set to 120.000 with a scan range of
214 300-1600 *m/z*. Data evaluation was done on the original mass spectra comparing with predicted
215 masses of corrinoids (Supplementary Table S2, for cobalamin standards see Supplementary Figure
216 S3).

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

218 Field emission-scanning electron microscopy (FE-SEM) was performed with co-cultures of *S.*
219 *multivorans* and *D. mccartyi* strains BTF08 and 195. Cells of 5 mL culture were incubated for 15 min
220 with 2.5% glutaraldehyde and pre-fixed for 2 h on poly-L-lysine coated coverslides (12 mm, Fisher
221 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

252 extracted, desalted using C18 ZipTip columns (Merck Millipore, Darmstadt, Germany) and
253 resuspended in 0.1% (v/v) formic acid before LC–MS/MS analysis.

254 Proteolytic lysates were separated using an Ultimate 3000 RSLCnano liquid chromatographic
255 instrument (Thermo Scientific, Germany). Mass spectrometry was performed on an Orbitrap Fusion
256 mass spectrometer (Thermo Scientific, San Jose, CA, USA) coupled to a TriVersa NanoMate (Advion,
257 Ltd., Harlow, UK). Samples of 5 µL were loaded onto a trapping column (Acclaim PepMap100 C18,
258 75 µm × 2 cm, Thermo Scientific) using 96% eluent A (0.1% formic acid) and 4% eluent B (0.08%
259 formic acid, 80% acetonitrile) at a flow rate of 5 µL min^{-1} and separated via a 25 cm analytical column
260 (Acclaim PepMap100 C18, 75 µm × 25 cm, Thermo Scientific) at 35°C using a constant flow rate of
261 300 nL/min. Peptide separation was achieved by applying a linear gradient of eluent B from 4% to
262 50% within 100 min. Full MS scans were measured in the Orbitrap mass analyzer within the mass
263 range of 400-1,600 *m/z* at 120,000 resolution using an automatic gain control (AGC) target of $4x10^5$
264 and maximum fill time of 60 ms. The MS instrument measured in data-dependent acquisition (DDA)
265 mode using the highest intense ion (top speed, 3 sec cycle time). Positive ion charge states between 2
266 and 7 were selected for MS/MS. Precursor masses for MS/MS were selected based on highest
267 intensity and excluded from further MS/MS for 30 s to prevent redundancy in MS/MS acquisition. After
268 higher energy collisional (HCD) fragmentation at normalized collision induced energy of 30%, fragment
269 masses were scanned in the Orbitrap mass analyzer at a resolution of 15,000 with $5x10^4$ AGC target
270 and a maximum injection time of 150 ms.
271 LC-MS/MS data were analyzed using Proteome Discoverer (v2.1, Thermo Scientific). MS/MS spectra
272 were searched against a combination of a *S. multivorans* (3,233 non-redundant protein-coding
273 sequences, downloaded January 2017 from NCBI GenBank, accession number CP007201.1) and a
274 *D. mccartyi* BTF08 database (1,535 non-redundant protein-coding sequences, downloaded December
275 2016 from NCBI, accession number CP004080.1). A “common repository of adventitious proteins
276 database” (cRAP) was integrated to ensure correct protein identifications. The SEQUEST HT
277 algorithm was used with the following settings: trypsin as cleavage enzyme, oxidation on methionine
278 as dynamic and carbamidomethylation on cysteine as static modification, up to two missed cleavages,
279 precursor mass tolerance set to 10 ppm and fragment mass tolerance to 0.02 Da, respectively. Only
280 peptides with a false discovery rate (FDR) 1%, XCorr ≥2, q-value and the posterior error probability
281 (PEP) ≤0.01 were considered as identified (Supplementary Excel File S1). Quantification of proteins

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, ggbiplot, 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 *c*DCE 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: ε_C = -0.4 ± 0.3 ‰; *S. multivorans* pure culture: ε_C = -2.0 ± 0.4 ‰, *D. mccartyi* BTF08: ε_C = -5.0 to -9.0 ‰ (Franke *et al*., unpublished)). The fast dechlorination of PCE to *c*DCE 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 *c*DCE (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 to5.9:1 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 *c*DCE 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 *c*DCE was obtained (~30 µmol/bottle/day) (Figure 2B). No further dechlorination of *c*DCE to VC or ethene was detected, indicating that *S. multivorans* alone was responsible for the dechlorination. When *c*DCE dechlorination stalled in the Sm/BTF08 co-culture, 1 µM 5,6-dimethylbenzimidazole (DMB) was added, which resulted in subsequent *c*DCE dechlorination (Figure 2C). Ethene production rates from *c*DCE (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 B_{12} amendment (-B_{12}) dechlorinated PCE to VC, although at low rates (Figure 2E). After 35 days, PCE was re-fed, and *c*DCE 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 -B_{12} was [Ade]NCba (Supplementary Figure S5). No significant increase in *c*DCE 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 *c*DCE to VC, we amended a pure culture with [Ade]NCba isolated from *S. multivorans* (Supplementary Figure S7A). Dechlorination of 50 µmol *c*DCE to VC was detected in 22 days. *D. mccartyi* 195 pure cultures amended with [5-OMeBza]Cba showed slightly faster dechlorination and the *c*DCE 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

385 electron micrographs. In the FISH pictures, the sizes of the aggregates ranged from 50 to 100 µm in
386 diameter.

387 *S. multivorans* revealed a typical helical rod shaped cell structure and a size of 2 to 5 µm to 0.5 µm at
388 magnifications of around 10.000x and 20.000x in FE-SEM as described previously (Scholz-Muramatsu
389 et al 1995) (Figure 4, Supplementary Figure S10). However, the typical polar flagellum was only
390 observed in a few cells and several flagella seemed to be detached from cells, possibly a part of the
391 ECM (Figure 4, Supplementary Figure S10). *D. mccartyi* showed atypical cell morphology in the co-
392 cultures. Microscopic analysis of the pure culture revealed a disc-shaped irregular coccus of 0.5 µm
393 diameter (Figure 4a, Supplementary Figure S11), as previously described (Löffler et al 2013), whereas
394 the *D. mccartyi* strains in the co-culture showed 0.5 µm large barrel-like cells with a flattened cell pole
395 at one side and a ring-shaped septum (Figure 4, Supplementary Figure S10). FISH analysis of
396 isolated cells in the co-culture identified unequivocally both organisms and confirmed the presence
397 and unusual morphology of *D. mccartyi* (Supplementary Figure S12 and S13).

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

402 We applied a label-free shotgun proteomics approach to identify changes in protein abundance during
403 PCE dechlorination in pure and co-cultures (with and without vitamin B_{12}) with *D. mccartyi* BTF08 and
404 *S. multivorans*. Samples for proteomic analyses were taken after one re-feeding with PCE or *c*DCE
405 (pure *D. mccartyi* cultures) or 12 hours after the third three re-feeding with PCE (co-cultures). While
406 PCE, TCE and cDCE were the dominant chlorinated ethenes in the co-cultures, *c*DCE and VC were
407 the dominant chlorinated ethenes in the pure culture when harvested (Supplementary Figure S14).
408 The clustering approach nMDS based on quantified proteins revealed a significant separation between
409 pure and co-cultures (Dhc BTF08 proteins p=0.001, *Sm* proteins p=0.004), indicating a difference of
410 the physiology of both organisms depending on the cultivation and (Supplementary Figure S15). A
411 multi-level pattern analysis was applied to determine indicator proteins for a given condition (i.e. co-
412 culture vs. pure culture) to better understand the functional changes occurring during the
413 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 dechlorination of PCE to *c*DCE, 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 pili 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_{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 *S. multivorans* with *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, *S. multivorans* grows by organohalide respiration with lactate as electron donor. In this phase, *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 *D. mccartyi* with hydrogen as electron donor due to the lack of (or very low) hydrogen production by *S. multivorans* during respiratory growth. In the second phase of the co-culture,when PCE has been completely converted to *c*DCE after less than two days, *S. multivorans* grows via fermentation of lactate mainly to acetate, CO_2 and hydrogen. It has been shown earlier that, for thermodynamic reasons, lactate can only be utilized fermentatively by *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 *c*DCE 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). *c*DCE 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 *c*DCE 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 *c*DCE 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 *c*DCE 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 *c*DCE 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,

503 however, not be excluded from our data that [DMB]NCba can be used as well by *D. mccartyi* BTF08
504 for cDCE or VC dechlorination. The nucleotide loop cleavage is most likely mediated by the
505 adenosylcobinamide hydrolase CbiZ, as shown for CbiZ of *Rhodobacter sphaeroides* (Gray and
506 Escalante-Semerena 2009a, Gray and Escalante-Semerena 2009b). Of the two CbiZ encoded in the
507 genome of *D. mccartyi* BTF08, one was detected in the proteome and showed a higher abundance in
508 the co-culture compared to the pure culture. These results present evidence of the involvement of
509 CbiZ in exchanging the complete nucleotide loop in *D. mccartyi*.

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

522 The cultivation of *D. mccartyi* in association with *S. multivorans* led to the formation of cell aggregates,
523 which is a common characteristic of obligate syntrophic interactions often found in e.g. acetogenic and
524 methanogenic communities (Hulshoff Pol et al 2004, Ishii et al 2005, Stams et al 2012). FISH staining
525 confirmed the close association of both organisms and provided first insights into the organization and
526 spatial distribution of the organisms within aggregates in a dechlorinating mixed culture. Electron
527 microscopic analysis of the co-cultures revealed cells in close physical contact embedded in an ECM
528 consisting of extracellular polymer substances (EPS)-like structures and surrounding filaments
529 including also flagella detached from *S. multivorans* cells. The decrease of intermicrobial distances
530 and establishment of cell-to-cell contacts should lead to increased metabolite fluxes (e.g. hydrogen)
531 between species according to Fick's law (Schink and Thauer 1988), ultimately enhancing growth and
532 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 *c*DCE 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 *c*DCE 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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830 **Table 1: Dechlorination rates** of different co-culture set-ups *of S. multivorans* and *D. mccartyi* BTF08 or 195 to
831 the corresponding end product VC or ethene. n. a. - not applied.

		Dechlorination rate (µmol day^{-1} bottle^{-1})		
		1st dose PCE	2nd dose PCE	3rd dose PCE
Organism(s)	**Electron donor**			
With vitamin B_{12}				
***D. mccartyi* BTF08**	H_2	0.9 ± 0.03	1.4 ± 0.09	n.a.
***D. mccartyi* 195**	H_2	1.5 ± 0.04	3.3 ± 0.17	n.a.
Sm/BTF08	Lactate	4.1 ± 0.2	11.6$^{\#}$	11.6$^{\#}$
Sm/195	Lactate	4.8 ± 0.1	11.6$^{\#}$	11.6$^{\#}$
Without vitamin B_{12}				
Sm/BTF08	Lactate	-	-	-
Sm/195	Lactate	0.9 ± 0.004	3.6$^{\#}$	3.6$^{\#}$
Without vitamin B_{12} + DMB				
Sm/BTF08	Lactate	3.6 ± 0.2	8.0$^{\#}$	8.0$^{\#}$
Sm/195	Lactate	0.8 ± 0.05	4.3$^{\#}$	4.3$^{\#}$

832 $^{\#}$, no standard deviation, since the PCE values for re-feeding are theoretical values of the amount PCE added to
833 the culture and were not measured.
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Figure 1: Dechlorination of chlorinated ethenes, growth and metabolite analysis of *S. multivorans*/*D. mccartyi* co-cultures with vitamin B_{12} 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). ∑ - mass balance; sum of PCE, TCE, *cis*-DCE, VC and ethene.

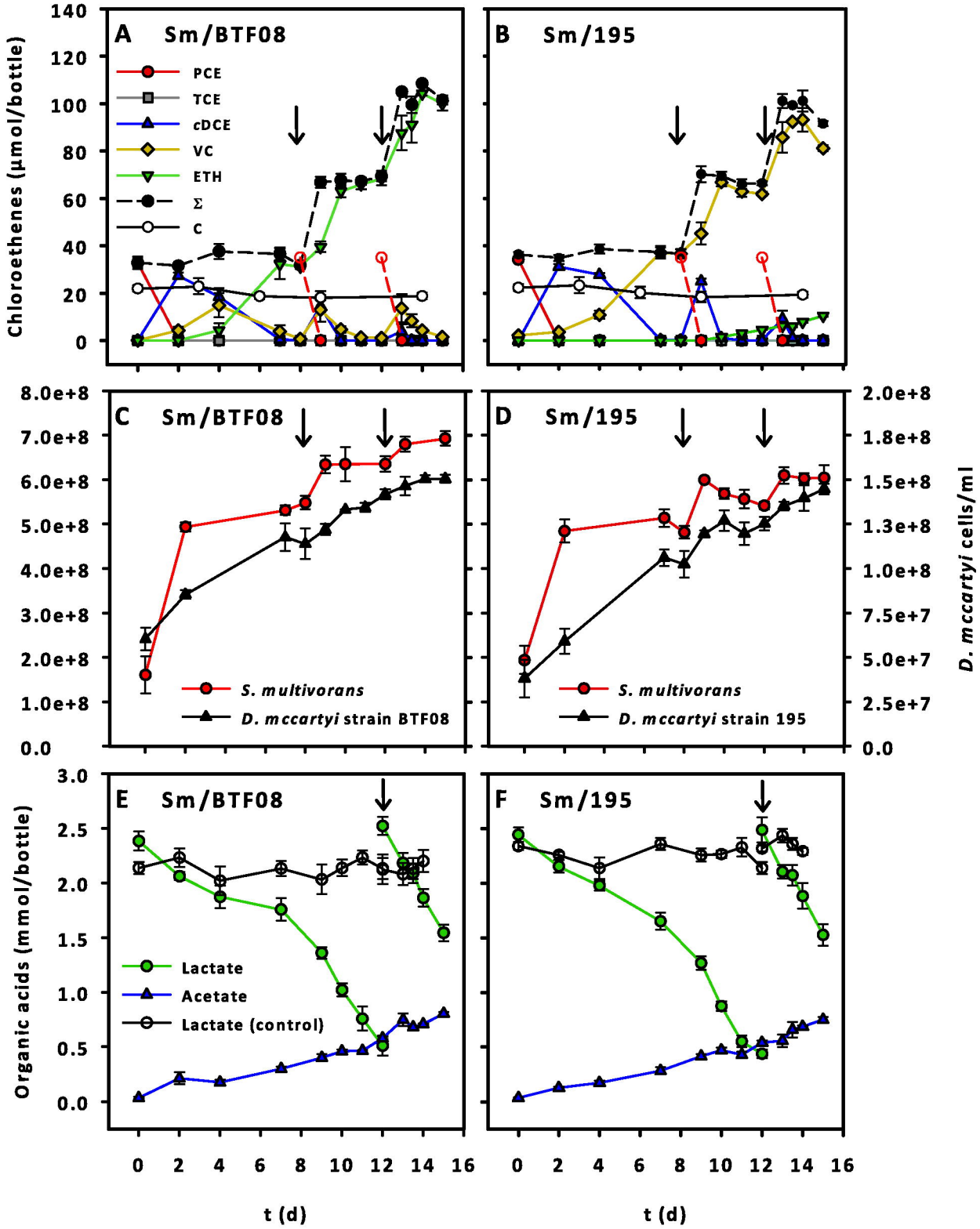
Figure 2: Dechlorination of *S. multivorans*/*D. mccartyi* co-cultures without addition of vitamin B_{12}. (A) Strain BTF08 with *c*DCE as electron acceptor (negative control). (B) Sm/BTF08 with PCE as the electron acceptor. (C) Sm/BTF08 with PCE as electron acceptor and amendment of 1 µM DMB. (D) Strain 195 with *c*DCE 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. ∑ - mass balance; sum of PCE, TCE, *c*DCE, VC and ethene.

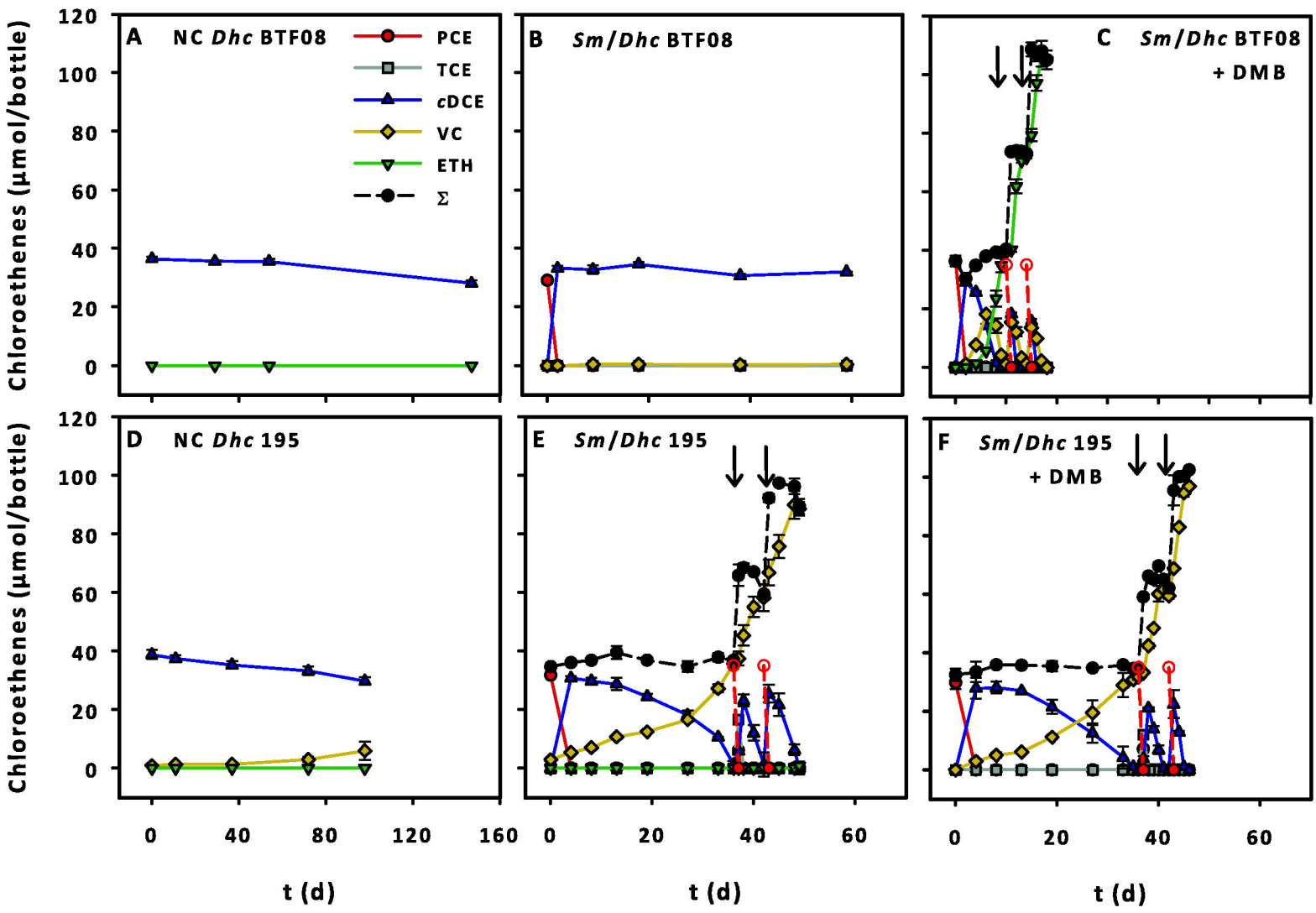
Figure 3: Microscopic analysis of cell aggregates in co-cultures of *S. multivorans* and *D. mccartyi* strain 195 and BTF08. (A) Serum bottle (200 mL) of a Sm/BTF08 co-culture showing cell aggregates. (B) Light microscopic image of a Sm/BTF08 aggregate. (C, D) Scanning electron micrographs of an aggregate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

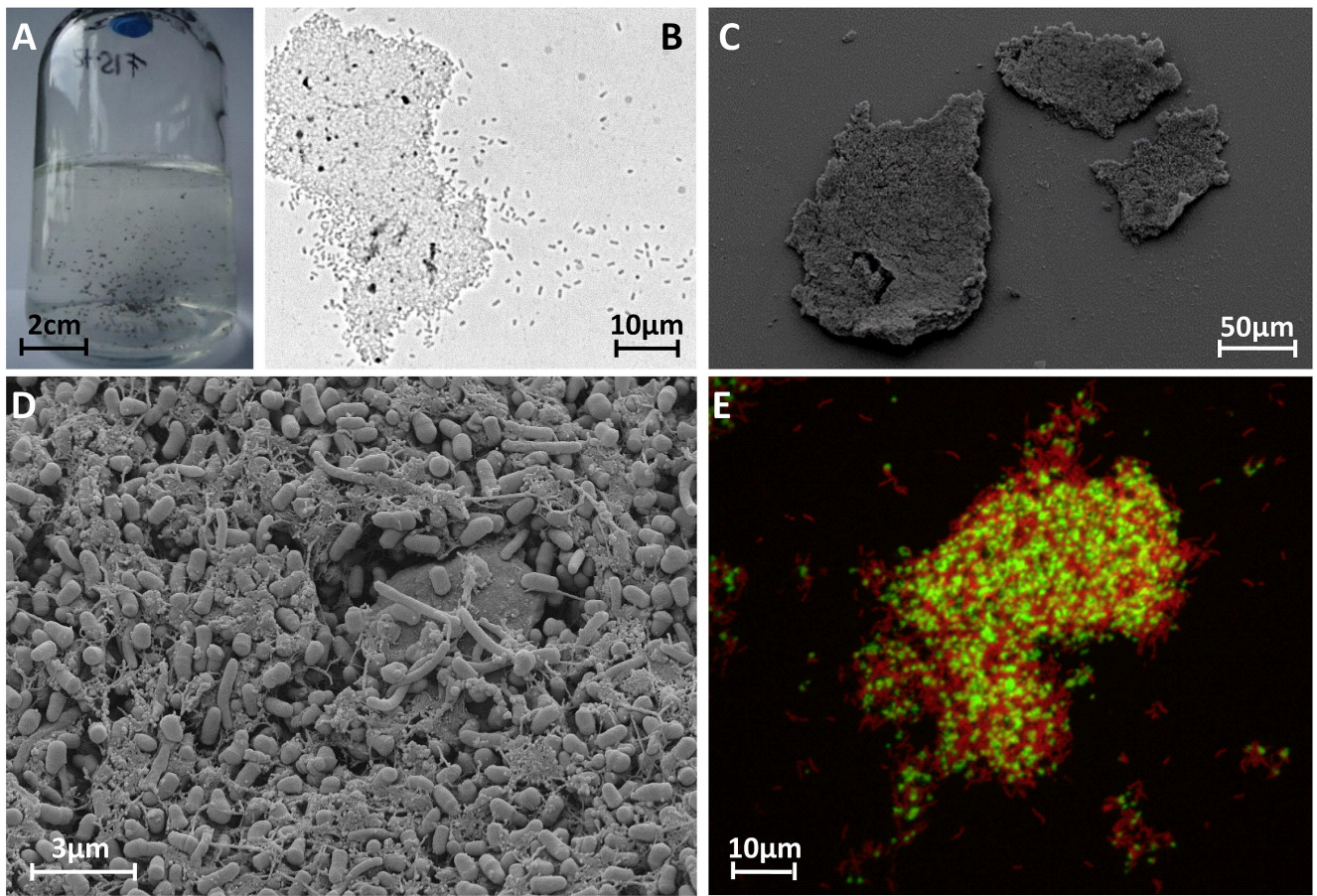
Figure 4: Different cell morphologies of *D. mccartyi* strain BTF08 cells. In pure culture (A) and co-culture with *S. multivorans* (B, C). White arrows indicate flagella and yellow arrows indicate ring-shaped septum.

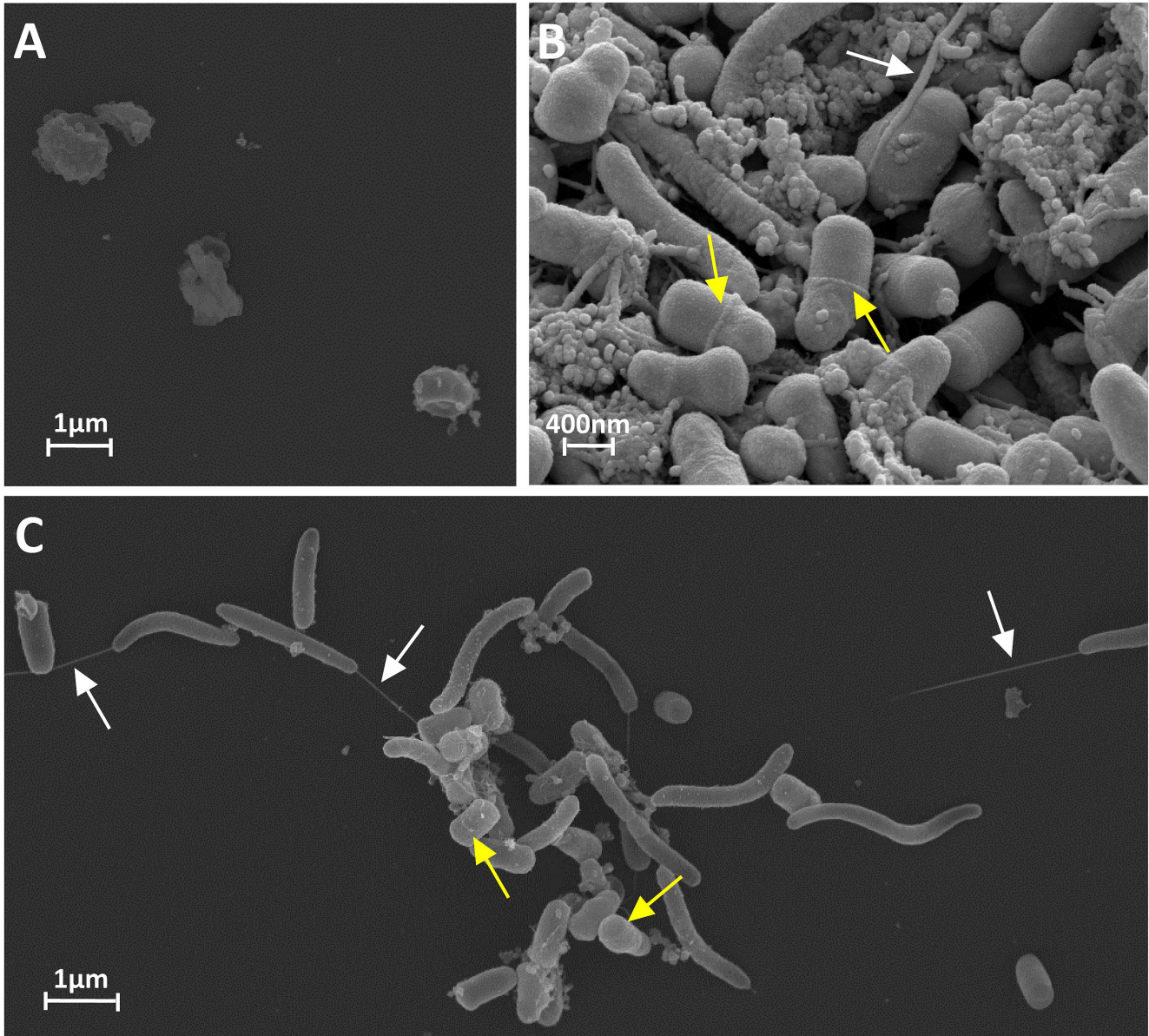
Figure 5: Protein abundances of reductive dehalogenases and significant indicator proteins of Dhc BTF08 in pure and co-cultures. Average protein abundance values under different cultivation conditions are shown. Abundances represent log10 fold changes of the median, scaled to zero. The median of all 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.

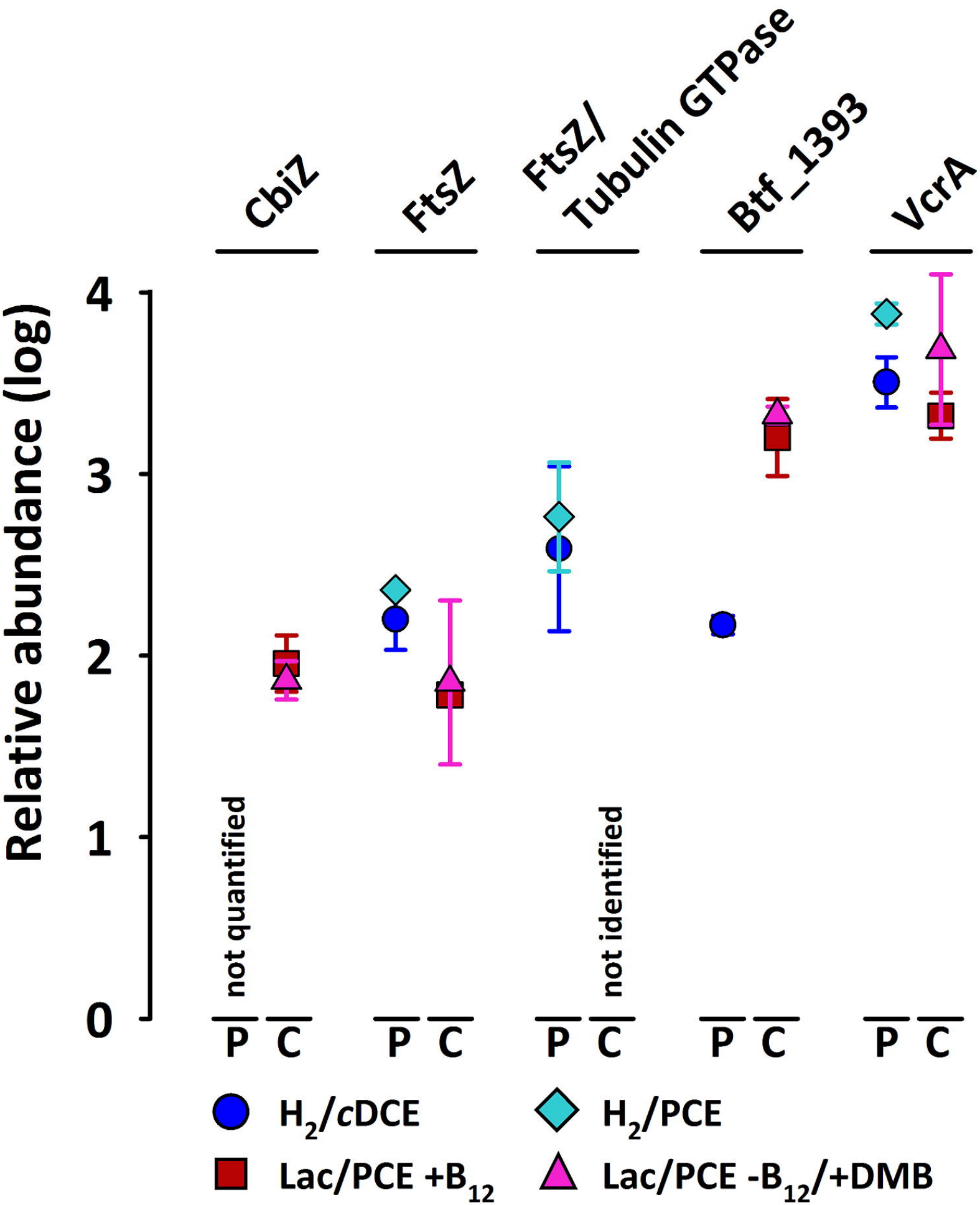
Figure 6: Interspecies metabolite transfer of *Sulfurospirillum multivorans* and *Dehalococcoides mccartyi*. PCE is dechlorinated to *c*DCE by *S. multivorans* with electrons from lactate oxidation. After depletion of PCE, *S. multivorans* switches to fermentatative metabolism, thereby generating hydrogen which is consumed by *D. mccartyi* as electron donor. The electron acceptor for *D. mccartyi* is *c*DCE and is further dechlorinated to ethene or VC by *D. mccartyi*. *S. multivorans* synthesizes norpseudo B_{12} ([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.

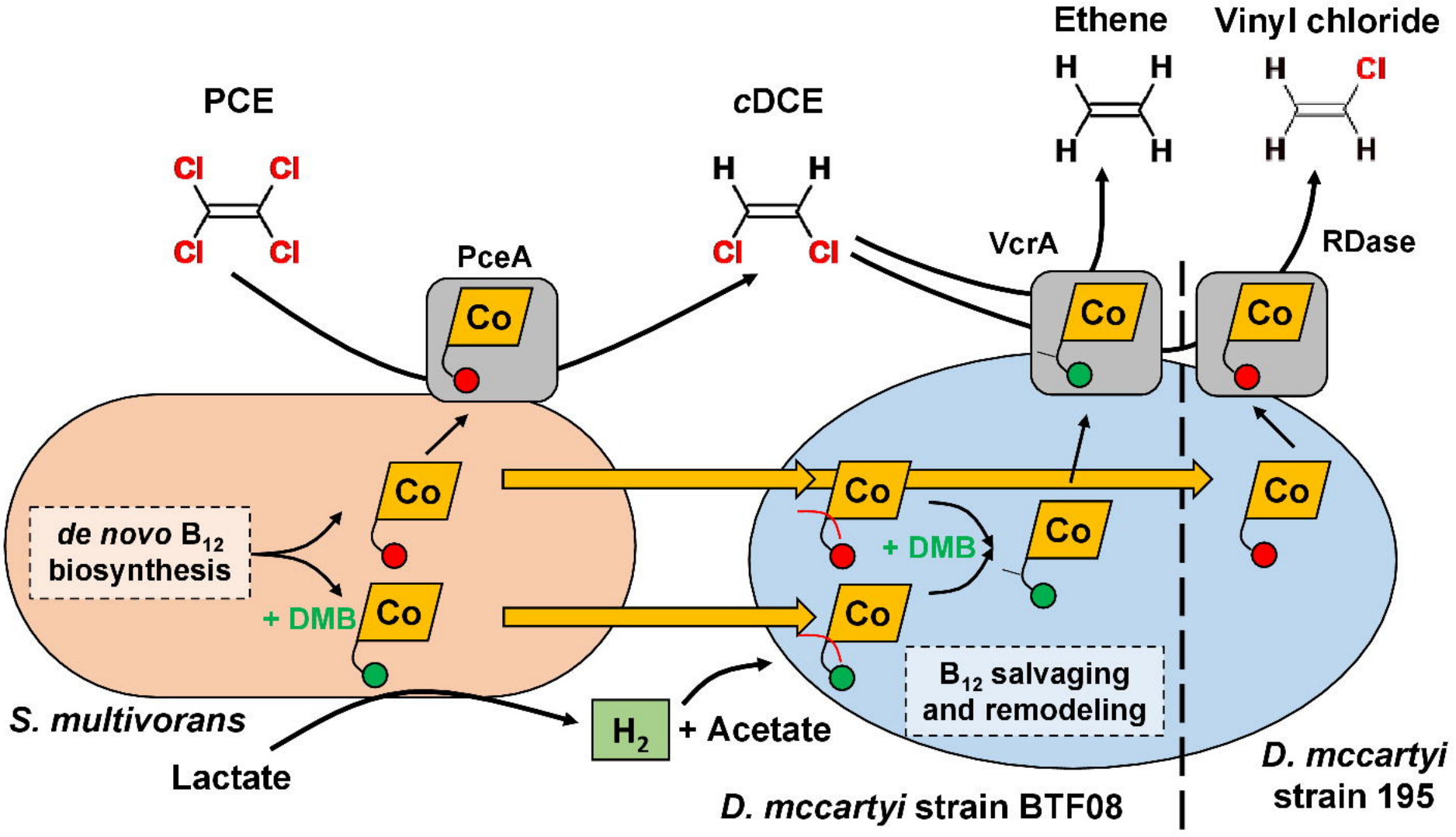

A Sm/BTF08
B Sm/195
C Sm/BTF08
D Sm/195
E Sm/BTF08
F Sm/195
PCE
TCE
cDCE
VC
ETH
Σ
C
Chloroethenes (µmol/bottle)
S. multivorans cells/ml
D. mccartyi cells/ml
S. multivorans
D. mccartyi strain BTF08
D. mccartyi strain 195
Organic acids (mmol/bottle)
Lactate
Acetate
Lactate (control)
t (d)


A NC Dhc BTF08
PCE
TCE
cDCE
VC
ETH
Σ
B Sm/Dhc BTF08
C Sm/Dhc BTF08
+ DMB
D NC Dhc 195
E Sm/Dhc 195
F Sm/Dhc 195
+ DMB
Chloroethenes (µmol/bottle)
t (d)


A
2cm
B
10µm
C
50µm
D
3µm
E
10µm


A
1µm
B
400nm
C
1µm


CbiZ
FtsZ
FtsZ/ Tubulin GTPase
Btf_1393
VcrA
Relative abundance (log)
4
3
2
1
0
not quantified
not identified
P C P C P C P C P C
H2/cDCE
H2/PCE
Lac/PCE +B12
Lac/PCE -B12/+DMB


Ethene
Vinyl chloride
PCE
cDCE
VcrA
RDase
PceA
Co
de novo B12 biosynthesis
+ DMB
+ DMB
B12 salvaging and remodeling
S. multivorans
Lactate
H2
+ Acetate
D. mccartyi strain BTF08
D. mccartyi strain 195