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Unexpected Specificity of Interspecies Cobamide Transfer from *Geobacter* spp. to Organohalide-Respiring *Dehalococcoides mccartyi* Strains

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Dehalococcoides mccartyi **strains conserve energy from reductive dechlorination reactions catalyzed by corrinoid-dependent reductive dehalogenase enzyme systems.** ***Dehalococcoides*** **lacks the ability for** ***de novo*** **corrinoid synthesis, and pure cultures require the addition of cyanocobalamin (vitamin B_{12}) for growth. In contrast,** ***Geobacter lovleyi*****, which dechlorinates tetrachloroethene to** ***cis*****-1,2-dichloroethene (*****cis*****-DCE), and the nondechlorinating species** ***Geobacter sulfurreducens*** **have complete sets of cobamide biosynthesis genes and produced 12.9 ± 2.4 and 24.2 ± 5.8 ng of extracellular cobamide per liter of culture suspension, respectively, during growth with acetate and fumarate in a completely synthetic medium.** ***G. lovleyi-D. mccartyi*** **strain BAV1 or strain FL2 cocultures provided evidence for interspecies corrinoid transfer, and** ***cis*****-DCE was dechlorinated to vinyl chloride and ethene concomitant with** ***Dehalococcoides*** **growth. In contrast, negligible increase in** ***Dehalococcoides*** **16S rRNA gene copies and insignificant dechlorination occurred in** ***G. sulfurreducens-D. mccartyi*** **strain BAV1 or strain FL2 cocultures. Apparently,** ***G. lovleyi*** **produces a cobamide that complements** ***Dehalococcoides*****' nutritional requirements, whereas** ***G. sulfurreducens*** **does not. Interestingly,** ***Dehalococcoides*** **dechlorination activity and growth could be restored in** ***G. sulfurreducens-Dehalococcoides*** **cocultures by adding 10 μM 5′,6′-dimethylbenzimidazole. Observations made with the** ***G. sulfurreducens-Dehalococcoides*** **cocultures suggest that the exchange of the lower ligand generated a cobalamin, which supported** ***Dehalococcoides*** **activity. These findings have implications for** ***in situ*** **bioremediation and suggest that the corrinoid metabolism of** ***Dehalococcoides*** **must be understood to faithfully predict, and possibly enhance, reductive dechlorination activities.**

Members of the genus of *Dehalococcoides* are strictly hydrogenotrophic, organohalide-respiring bacteria and are recognized as the key players to achieve detoxification at sites contaminated with chlorinated ethenes (13, 18). *Dehalococcoides* genomes harbor multiple genes encoding reductive dehalogenases (RDases) that catalyze the reductive dehalogenation of a range of halogenated compounds (1, 2, 15, 20, 21, 27). For example, the *pceA* and *tceA* genes of *Dehalococcoides mccartyi* strain 195 encode RDases that catalyze reductive dechlorination of tetrachloroethene (PCE) to trichloroethene (TCE) and of TCE to vinyl chloride (VC) and ethene, respectively (20, 21, 33). The VC-respiring *D. mccartyi* strain BAV1 cannot utilize PCE or TCE but possesses *bvcA*, enabling this strain to grow with dichloroethenes and VC as electron acceptors (10, 11). Several RDases of organohalide-respiring strains belonging to the genera *Desulfomonile*, *Dehalococcoides*, *Dehalobacter*, *Desulfitobacterium*, and *Sulfurospirillum* were, at least partially, characterized (22, 26, 28, 29). Except for the 3-chlorobenzoate RDase of *Desulfomonile tiedjei* strain DCB-1 (29), all characterized RDases of organohalide-respiring bacteria contain a cobalamin or cobamide (i.e., a cobalt-containing cyclic tetrapyrrole) cofactor, which is involved in electron transfer and the reduction of the chloro-organic electron acceptor (6, 34). *Dehalococcoides* isolates grow and perform reductive dechlorination in completely synthetic, defined medium as long as cyanocobalamin (vitamin B_{12}) is provided (4). Genome sequence analysis revealed that *Dehalococcoides* strains cannot synthesize the corrin ring structure *de novo* nor introduce a cobalt atom to the tetrapyrrole ring; however, *Dehalococcoides* strains possess both the archaeal and bacterial salvage pathways to scavenge (incomplete) corrinoids from the environment (44, 45). Many anaerobic *Bacteria* and *Archaea* share the ability to synthesize these complicated, cobalt-containing cyclic tetrapyrrole cofactors *de novo*. It is generally assumed that excretion or release following cell lysis maintains a corrinoid flux that fulfills the nutritional requirements of corrinoid-auxotrophs, including *Dehalococcoides*. For example, methanogens and acetogens synthesize methylcobamides or other vitamin B_{12} analogues to assemble functional corrinoid-dependent methyltransferases that catalyze methyl transfer reactions crucial for methanogenesis and acetogenesis (19, 23). Sulfate reducers, such as *Desulfobacterium autotrophicum* and *Desulfobulbus propionicus*, synthesize 5′-methylbenzimidazolyl-cobamide, a required cofactor in the acetyl coenzyme A pathway for CO_2 fixation and acetate oxidization (17).

Geobacter spp. are ubiquitous in anoxic sediment and subsurface environments, and the genome sequences of several isolates have been obtained (3). *Geobacter sulfurreducens* strain PCA (GenBank accession no. AE017180.1) and *Geobacter lovleyi* strain SZ (CP001089.1) possess all of the required genes to synthesize cobamide(s) (25, 43). Both of these species share the hallmark

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features of the *Geobacter* genus and are acetate-oxidizing, dissimilatory metal reducers, but only *Geobacter lovleyi* strain SZ performs organohalide respiration and conserves energy from PCE and TCE to *cis*-DCE dechlorination (39). *Geobacter* spp. and *Dehalococcoides mccartyi* strains co-occur in sediments and subsurface environments (43), but it is unknown in what capacity *Geobacter* spp. fulfill *Dehalococcoides*' corrinoid requirements. The objective of the present study was to explore whether cobamides synthesized by an organohalide-respiring and a nondechlorinating *Geobacter* species support the growth of corrinoid-requiring *Dehalococcoides* strains.

MATERIALS AND METHODS

Chemicals. Chlorinated compounds were of >99% purity. PCE was purchased from ACROS Organics (Morris Plains, NJ), TCE was purchased from Fisher (Pittsburgh, PA), and *cis*-DCE, VC, and ethene (99.5%) were purchased from Sigma-Aldrich-Fluka (St. Louis, MO).

Cultures and growth conditions. Pure cultures of *Dehalococcoides mccartyi* strain BAV1 (ATCC BAA-2100 = JCM 16839 = KCTC 5957) and strain FL2 (ATCC BAA-2098 = DSM 23585 = JCM 16840 = KCTC 5959) were maintained in reduced, bicarbonate-buffered mineral salts medium as described previously (11, 12). In brief, acetate and hydrogen (10 to 20% of headspace) served as carbon source and electron donor, respectively, and the medium received 25 μg of vitamin B_{12} (cyanocobalamin) liter^{-1}. Neat TCE or *cis*-DCE was added to serve as an electron acceptor for strains FL2 and BAV1, respectively. *G. lovleyi* strain SZ (DSM 17278) and *G. sulfurreducens* strain PCA (DSM 12127) were maintained in vitamin B_{12}-free, reduced, bicarbonate-buffered mineral salts medium amended with acetate (5 mM) as electron donor and PCE (0.5 mM, aqueous-phase concentration) or fumarate (1.5 or 10 mM) as electron acceptors (39). All pure cultures and defined cocultures were established in 160-ml glass serum bottles containing 100 ml of defined mineral salts medium with a N_2-CO_2 (80/20 [vol/vol]) headspace and incubated statically at 30°C in the dark.

Cobamide production in *Geobacter* cultures. Cobamide production in *G. lovleyi* and *G. sulfurreducens* cultures was quantified in medium supplemented with fumarate (1.5 mM and 10 mM for *G. lovleyi*; 10 mM for *G. sulfurreducens*) as the electron acceptor. Extracellular cobamides were also measured in *G. lovleyi* cultures grown with 0.5 mM PCE as the electron acceptor.

***Geobacter-Dehalococcoides* cocultures.** Triplicate cocultures of *G. lovleyi* or *G. sulfurreducens* and *D. mccartyi* strain BAV1 or strain FL2 were established in culture vessels containing vitamin B_{12}-free medium, and 10 ml of hydrogen was provided as the electron donor for *Dehalococcoides*. (i) For the *G. lovleyi-Dehalococcoides* cocultures Glov-BAV1 and Glov-FL2, neat PCE (~0.5 mM aqueous-phase concentration) and 1.5 mM fumarate were added to each vessel as the electron acceptors for *G. lovleyi*. The PCE dechlorination product *cis*-DCE generated by *G. lovleyi* was subsequently available as an electron acceptor for *Dehalococcoides* strains BAV1 and FL2. (ii) For the *G. sulfurreducens-Dehalococcoides* cocultures Gsul-BAV1 and Gsul-FL2, neat *cis*-DCE and TCE, respectively, were added to achieve aqueous phase concentrations of ~0.5 mM. Cells for inoculation were prepared inside an anoxic chamber (Coy Laboratory Products, Grass Lake, MI) by centrifuging 3-ml culture suspensions at 14,000 × *g* for 10 min at room temperature, decanting the supernatant, and then suspending the cell pellets in vitamin B_{12}-free medium to minimize corrinoid carryover. Cocultures amended with 25 μg of vitamin B_{12} liter^{-1} were constructed the same way to serve as positive controls. Cultures of *D. mccartyi* strain BAV1 and strain FL2 without vitamin B_{12} additions served as negative controls.

DNA extraction and quantification of 16S rRNA genes. Cells were harvested by vacuum filtration of 1-ml culture suspensions through 0.22-μm membrane filters (Millipore GVWP02500), and genomic DNA was extracted from the 25-mm membranes by using the MoBio soil DNA isolation kit (MO BIO, Carlsbad, CA) according to the manufacturer's protocol. The primer set Dhc1200F/Dhc1271R and the FAM-BHQ1 Dhc1240 probe were used in quantitative real-time PCR (qPCR) to enumerate *Dehalococcoides* 16S rRNA gene copies. The details for these qPCR procedures, including standard curve preparation, have been reported (30).

Cobamide measurements. Cobamides were quantified in 96-well microtiter plates based on a microbiological assay using the cobamide-auxotroph *Lactobacillus delbrueckii* subsp. *lactis* (ATCC 7830) as the test organism (32, 35, 41). Liquid samples were withdrawn from pure cultures and cocultures. Cells were removed by centrifugation at 14,000 × *g* for 1 min, and the supernatants were passed through 0.22-μm-pore-size membrane filters. Cell-free supernatant (150 μl) was diluted 2- to 20-fold with water and distributed into individual microtiter plate wells, which were filled with 150 μl of double-strength vitamin B_{12} assay medium (Difco, Franklin Lakes, NJ) and an initial *L. delbrueckii* cell titer of 1.5×10^4 to 2.0×10^4 ml^{-1}, as determined by plate counting. To prepare vitamin B_{12}-free inocula, a −80°C stock culture of *L. delbrueckii* was grown in vitamin B_{12} inoculum broth (Difco) overnight at 37°C. The cells were harvested at 14,000 × *g* for 2 min and washed with sterile, deionized water three times. The cells were suspended in double-strength vitamin B_{12} assay medium diluted with an equal amount of sterile, deionized water and then incubated at 37°C for 3 h to deplete carryover B_{12} from the inoculum broth (42). The microtiter plate was sealed with an adhesive optical cover and incubated at 37°C in the dark. After 24 h of incubation, the optical density in each well was recorded at 630 nm using a microtiter plate reader (BioTek, Winooski, VT). A standard curve was included on each plate and was generated by adding known concentrations of vitamin B_{12}. This microbiological assay had a linear range from 5 to 50 ng of vitamin B_{12} liter^{-1} with a detection limit of 2 ng liter^{-1} (see Fig. S1 in the supplemental material). Cobamides measured using this microbiological approach were reported as vitamin B_{12} equivalents.

Analytical methods. Chlorinated ethenes and ethene were analyzed using an HP 6890 gas chromatograph equipped with a flame ionization detector and a DB-624 capillary column (60 m by 0.32 mm with a film thickness of 0.18 μm) (40). Fumarate and succinate were analyzed using an Agilent 1200 series high-performance liquid chromatography system. Samples (475 μl) were acidified with 25 μl of 1 M H_2SO_4, separated on an Aminex HPX-87H column (Bio-Rad, Hercules, CA) with 4 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 ml min^{-1}, and quantified using a multiple wavelength detector at 210 nm.

RESULTS

Cobamide production in *Geobacter* spp. pure cultures. Both *Geobacter* cultures were transferred and maintained in completely synthetic, vitamin B_{12}-free medium for more than 3 years, indicating that both species are capable of *de novo* cobamide biosynthesis. The microbiological assay demonstrated extracellular cobamide production in *G. lovleyi* cultures growing with PCE (0.5 mM in aqueous phase) or fumarate (1.5 mM) as the electron acceptor (Fig. 1A and B). After complete reduction of PCE to *cis*-DCE and fumarate to succinate, cobamide concentrations of 12.9 ± 2.4 and 24.2 ± 7.1 ng liter^{-1}, respectively, were measured in the culture medium. Extracellular cobamide levels continued to rise following electron acceptor consumption, and at day 23 (i.e., about 19 days after PCE or fumarate had been consumed), the cobamide concentrations increased to 34.5 ± 3.3 and 79.3 ± 7.4 ng liter^{-1}, respectively. *G. lovleyi* cultures that received 10 mM fumarate produced 39.3 ± 4.2 ng of extracellular cobamide liter^{-1} following electron acceptor consumption. *G. sulfurreducens* cultures produced similar amounts of extracellular cobamide(s), and 24.2 ± 5.8 ng of cobamide liter^{-1} was measured after a 64-h incubation period (Fig. 1C).

Dechlorination in *Geobacter-Dehalococcoides* cocultures. In

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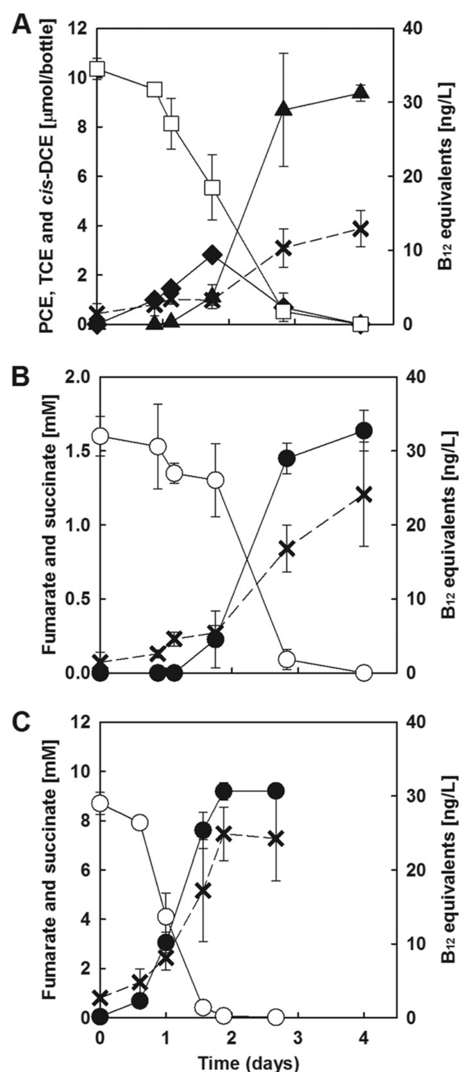

FIG 1 Cobamide production in *Geobacter* pure cultures. *G. lovleyi* was grown in 60-ml serum bottles containing 40 ml of medium with PCE (0.5 mM in aqueous phase) (A) or fumarate (1.5 mM) (B) as the electron acceptor. (C) Cobamide production in *G. sulfurreducens* pure cultures with fumarate (10 mM) as the electron acceptor. Error bars represent standard deviations and are not shown if they are smaller than symbols. Symbols: □, PCE; ◆, TCE; ▲, *cis*-DCE; ○, fumarate; ●, succinate; ×, cobamide(s) reported as vitamin B_{12} equivalents.

the *G. lovleyi-D. mccartyi* (Glov-BAV1 and Glov-FL2) cocultures, PCE was stoichiometrically dechlorinated to 42.4 ± 1.2 μmol of *cis*-DCE after 7 to 10 days, independent of the addition of vitamin B_{12}. In the positive control cocultures amended with 25 μg of vitamin B_{12} liter^{-1}, *cis*-DCE was further dechlorinated to ethene and a VC-ethene mixture by strain BAV1 and strain FL2, respectively (Fig. 2A and D), demonstrating that both *Dehalococcoides* strains were able to dechlorinate biologically produced *cis*-DCE and to grow during cocultivation with *G. lovleyi*. Some dechlorination occurred in *Dehalococcoides* pure culture controls amended with *cis*-DCE but lacking vitamin B_{12}. Up to 36% (BAV1 cultures) and 10% (FL2 cultures) of the initial amounts of *cis*-DCE were reduced to VC, but all dechlorination activities ceased after 49 days, and no ethene was formed (Fig. 2B and E). In cocultures that did not receive vitamin B_{12}, *cis*-DCE was readily dechlorinated albeit at lower rates (0.46 to 0.62 μmol/bottle/day) compared to the replicate cocultures (2.5 to 3.03 μmol/bottle/day) that were amended with vitamin B_{12} (Fig. 2C and F). All *cis*-DCE produced from PCE was dechlorinated to VC and ethene (27.42 ± 0.36 and 9.31 ± 1.40 μmol per bottle, respectively) in the Glov-BAV1 cocultures after 100 days (Fig. 2C). In the Glov-FL2 cocultures, >90% of the *cis*-DCE was dechlorinated to VC (Fig. 2F), whereas <10% of the *cis*-DCE was dechlorinated to VC in the strain FL2 negative control incubations (Fig. 2E).

Very limited dechlorination occurred in the Gsul-BAV1 and Gsul-FL2 cocultures amended with *cis*-DCE and TCE, respectively. After a 66-day incubation period, 21.7% ± 3.6% of the *cis*-DCE was dechlorinated to VC in the Gsul-BAV1 cocultures, and 1.7% ± 0.1% of the TCE was dechlorinated to *cis*-DCE in the Gsul-FL2 cocultures (Fig. 3A and B). Similar amounts of dechlorination products were observed in the corresponding negative control cultures, in which 21.8% ± 4.2% of the *cis*-DCE and 2.2% ± 0.3% of the TCE were dechlorinated (see Fig. S2B and D in the supplemental material). The positive controls of *G. sulfurreducens-D. mccartyi* cocultures (i.e., cultures amended with vitamin B_{12}) reduced fumarate to stoichiometric amounts of succinate, *cis*-DCE was dechlorinated to ethene by strain BAV1 and TCE was dechlorinated to VC and ethene by strain FL2 (see Fig. S2A and C in the supplemental material).

Recovery of *Dehalococcoides* dechlorination activity in *G. sulfurreducens-Dehalococcoides* cocultures. Nondechlorinating Gsul-BAV1 and Gsul-FL2 cocultures (Fig. 3A and B) were amended with 10 μM 5′,6′-dimethylbenzimidazole (DMB), the lower nucleotide ligand in vitamin B_{12}. Complete reductive dechlorination of TCE to VC (29.36 ± 1.72 μmol per bottle) and ethene (5.23 ± 1.17 μmol per bottle) occurred in the Gsul-FL2 cocultures, and *cis*-DCE was reduced to stoichiometric amounts of ethene in the Gsul-BAV1 cocultures after 66 days (Fig. 3C and D). The addition of DMB to *Dehalococcoides* pure cultures lacking vitamin B_{12} did not result in dechlorination activity, and no inhibitory effects were observed at the DMB concentrations used in cultures amended with vitamin B_{12}.

Cobamide production in the cocultures. Immediately after inoculation, the microbiological assay did not detect cobamides in any of the cocultures that were not amended with vitamin B_{12}. Cobamide was detected after about 15 days and the amounts increased to maximum concentrations of 16.2 ± 2.0 ng liter^{-1} in the Glov-BAV1 cocultures and 26.1 ± 3.4 ng liter^{-1} in the Glov-FL2 cocultures (Fig. 2C and F). Extracellular cobamides accumulated to higher concentrations of 367 ± 41 ng liter^{-1} in the Gsul-BAV1 cocultures and 361 ± 80 ng liter^{-1} in the Gsul-FL2 cocultures (Fig. 3A and B). Lower cobamide concentrations were detected in the DMB-amended Gsul-BAV1 and Gsul-FL2 cocultures, and maximum concentrations of 138 ± 52 and 152 ± 50 ng liter^{-1} were measured, respectively (Fig. 3C and D).

Growth of *Dehalococcoides* in the cocultures. To corroborate *Dehalococcoides* growth, *Dehalococcoides* 16S rRNA genes were quantified in the cocultures (Table 1). In the Glov-BAV1 and Glov-FL2 cocultures, the *Dehalococcoides* cell numbers increased from (3.76 ± 1.16) × 10^6 and (7.33 ± 0.81) × 10^5 (i.e., cells introduced with the inoculum) to (8.05 ± 0.93) × 10^7 and (2.85 ± 0.61) × 10^7 gene copies per ml, respectively. Significant increases in *Dehalococcoides* 16S rRNA genes in the Gsul-BAV1 and Gsul-FL2 cocultures were only detected after the addition of DMB. *D. mccartyi* strain BAV1 and strain FL2 cell numbers increased from

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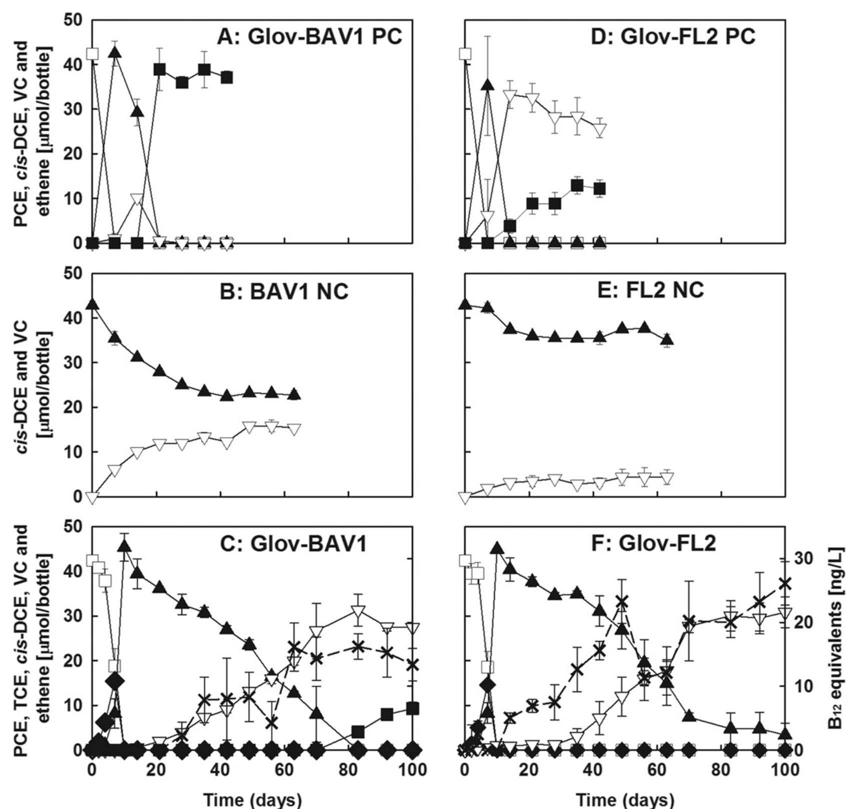

FIG 2 Dechlorination and extracellular cobamide production in *G. lovleyi* cocultures. (A) Glov-BAV1 positive control coculture with 25 μg of vitamin B_{12} liter^{-1}; (B) BAV1 negative control with *cis*-DCE as electron acceptor without vitamin B_{12}; (C) Glov-BAV1 coculture without B_{12} addition; (D) Glov-FL2 positive control with 25 μg of vitamin B_{12} liter^{-1}; (E) FL2 negative control with *cis*-DCE as electron acceptor; (F) Glov-FL2. Symbols: □, PCE; ◆, TCE; ▲, *cis*-DCE; ■, ethene; ▽, vinyl chloride; ×, cobamide(s) reported as vitamin B_{12} equivalents.

$(7.30 \pm 1.09) \times 10^6$ and $(7.50 \pm 2.70) \times 10^5$ (i.e., cells introduced with the inoculum) to $(3.13 \pm 0.48) \times 10^8$ and $(9.77 \pm 2.24) \times 10^7$ per ml, which corresponds to 42- and 129-fold increases. Negligible growth of *Dehalococcoides* was observed in Gsul-BAV1 and Gsul-FL2 cocultures that did not receive DMB, and BAV1 and FL2 cell numbers increased only slightly to $(1.13 \pm 0.14) \times 10^7$ and $(1.11 \pm 0.14) \times 10^6$ per ml, respectively, which corresponds to an increase of ~0.5-fold.

TABLE 1 Growth of *D. mccartyi* strains BAV1 and FL2 in coculture with *G. lovleyi* or *G. sulfurreducens*

Coculture[a]	*Dehalcococcoides* 16S rRNA gene copies/ml		Fold increase[c]
	Inoculum	Cell increase[b]	
Glov-BAV1	$(3.76 \pm 1.16) \times 10^6$	$(8.05 \pm 0.93) \times 10^7$*	20.4
Glov-FL2	$(7.33 \pm 0.81) \times 10^5$	$(2.85 \pm 0.61) \times 10^7$*	37.9
Gsul-BAV1			
DMB−	$(7.30 \pm 1.09) \times 10^6$	$(1.13 \pm 0.14) \times 10^7$†	0.55
DMB+	$(7.30 \pm 1.09) \times 10^6$	$(3.13 \pm 0.48) \times 10^8$†	41.8
Gsul-FL2			
DMB−	$(7.50 \pm 2.70) \times 10^5$	$(1.11 \pm 0.14) \times 10^6$†	0.48
DMB+	$(7.50 \pm 2.70) \times 10^5$	$(9.77 \pm 2.24) \times 10^7$†	129

[a] DMB−, DMB not added; DMB+, DMB added.
[b] *, after 90 days; †, after 66 days.
[c] Data were averaged from triplicates.

DISCUSSION

Corrinoid is a general term for compounds that contain a heterocyclic corrin tetrapyrrole ring such as vitamin B_{12} (i.e., cyanocobalamin) (31). Cobamides are corrinoids that consist of three parts, a corrin ring system containing a central, coordinated cobalt atom, an upper β-ligand moiety covalently bound to cobalt, and a lower α-ligand moiety noncovalently coordinated with the cobalt atom. Cobamides with a variety of lower ligands have been identified and are called cobalamins when DMB is the lower ligand (32). Typical cobalamins include methylcobalamin, adenosylcobalamin, hydroxocobalamin, and cyanocobalamin (vitamin B_{12}), which differ by their upper β-ligand moieties. Although the first three cobalamins listed occur naturally and are required for specific enzymatic functions, the last has a cyanide (CN) group as the upper β-ligand, which is an artifact introduced during purification; nevertheless, cyanocobalamin is commonly supplemented to corrinoid-auxotrophs indicating that many organisms, including organohalide-respiring *Dehalococcoides* strains, can substitute the upper CN ligand. Cobamides without the lower ligand are cobinamides, which are precursors of functional corrinoid cofactors (44).

In the current understanding of the reductive dechlorination process, a corrinoid cofactor is required to assemble functional RDase enzyme systems. It is therefore surprising that *Dehalococcoides* strains do not possess *de novo* pathways for assembly of the cobalt-containing cyclic tetrapyrrole cofactor essential for reduc-

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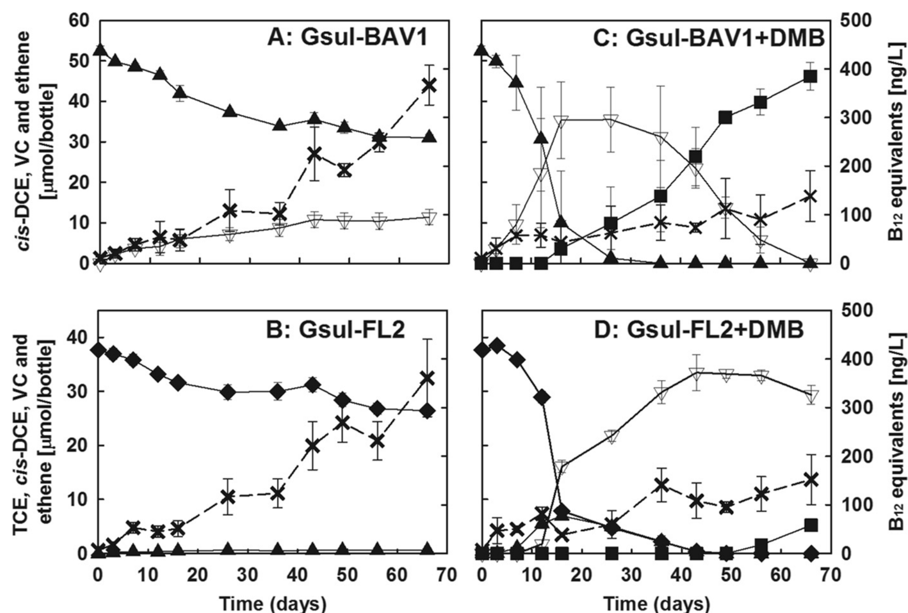

FIG 3 Dechlorination of *cis*-DCE and TCE by *D. mccartyi* strains BAV1 and FL2 in *G. lovleyi* cocultures without B_{12} amendment. (A) Gsul-BAV1 with *cis*-DCE as the electron acceptor; (B) Gsul-FL2 with TCE as the electron acceptor; (C) Gsul-BAV1 plus 10 μM DMB with *cis*-DCE as the electron acceptor; (D) Gsul-FL2 plus 10 μM DMB with TCE as the electron acceptor. Symbols: ◆, TCE; ▲, *cis*-DCE; ■, ethene; ▽, vinyl chloride; ×, cobamide(s) reported as vitamin B_{12} equivalents.

tive dechlorination and associated energy conservation. Assembly of cobalt-containing corrinoids is a complicated and energetically demanding process requiring substantial genetic information (>25 genes) (7). *D. mccartyi* strains rely on corrinoid salvage pathways to scavenge corrinoids from the environment to assemble functional RDases. The reasons why *Dehalococcoides* strains do not possess the biosynthetic machinery for a crucial cofactor are unclear; however, a sensible explanation is the energetic burden associated with *de novo* corrinoid biosynthesis. In pristine habitats, chloro-organic compounds are generally present in low concentrations (8), suggesting that strictly organohalide-respiring bacteria face energy limitations in most natural environments not impacted by anthropogenic contamination.

To quantify extracellular cobamides in the ng $liter^{-1}$ range, a sensitive microbiological assay was used (35, 41). Growth of the cobamide-auxotroph *Lactobacillus delbrueckii* subsp. *lactis* strain was proportional to the amount of cobamide(s) added to the assay medium, but no growth occurred with cobinamides (i.e., cobamides lacking the lower ligand) (32, 36). The assay measured cobamide increases during cultivation of *G. lovleyi* and *G. sulfurreducens*, demonstrating that both organisms synthesize cobamides. However, only *G. lovleyi* produced a cobamide that supported *Dehalococcoides* reductive dechlorination activity, suggesting that *G. sulfurreducens* assembled a cobamide with a different lower ligand. The observation that the addition of DMB to nondechlorinating *G. sulfurreducens*-*Dehalococcoides* cocultures restored the dechlorinating phenotype suggested that a cobalamin derivative (i.e., a cobamide with DMB as the lower ligand) is the cofactor required by *Dehalococcoides*. The characterization of the PCE RDases of *Dehalobacter restrictus* and *Sulfurospirillum multivorans* also suggested the involvement of cobamides, although the latter organism used pseudovitamin B_{12} with a minor modification in the side chain that connects the corrin ring and the lower ligand adenine (16). These findings suggest that cobamides with DMB or an analogue (e.g., adenine) as the lower ligands serve as cofactors in RDases of Gram-positives (e.g., *Dehalobacter*), Gram-negatives (e.g., *Sulfurospirillum*), and the organohalide-respiring *Chloroflexi*. Since the growth response of *L. delbrueckii* to different cobamides varies (32), the assay cannot accurately determine the actual cobamide concentrations in the *G. lovleyi* and the *G. sulfurreducens* cultures; however, the amounts of cobamides produced in the *Geobacter* pure cultures and the corresponding cocultures can be directly compared.

Many *Bacteria* and *Archaea* synthesize cobamides under oxic or anoxic conditions (31); however, a number of anaerobic microorganisms generate various cobamides by synthesizing DMB derivatives or non-benzimidazole-type nucleotide bases as the lower α-ligand (37). Subsurface microbial communities likely produce a variety of cobamides, and it has been assumed that a diverse corrinoid pool fulfills *Dehalococcoides*' nutritional requirements. Genes involved in DMB biosynthesis have remained elusive (31), but it is not uncommon that microorganisms take up different bases supplied in the growth medium and exchange lower α-ligands (32). Our findings suggest that *Geobacter* spp. produce distinct cobamides, and the observed exchange of the lower ligand in the *G. sulfurreducens* cocultures indicated that *Dehalococcoides* activity depends on specific forms of cobamides, such as cobalamins. Specifically, the organohalide-respiring species *G. lovleyi* produced a cobamide, likely a cobalamin, that supported *Dehalococcoides* activity, whereas the nondechlorinating *G. sulfurreducens* generated a cobamide with a non-DMB lower ligand. Both *Geobacter* spp. possess highly similar, homologous genes of the *de novo* cobamide biosynthesis pathway (see Table S1 in the supplemental material), but *G. sulfurreducens* apparently incorporates a different lower ligand, and *Dehalococcoides* cannot use the resulting cobamide. Cobamides with various lower

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ligands have been identified, and 5′-hydroxybenzimidazole and adenine are commonly found in methanogens, whereas 5′-methoxybenzimidazole, 5′-methoxy-6′-methylbenzimidazole, and *p*-cresol were described in acetogens such as *Clostridium* spp. and *Sporomusa* spp. (38). Cobamides synthesized by *Geobacter* spp. have not been characterized and our results indicate that *G. lovleyi* and *G. sulfurreducens* produce cobamides with different lower ligands.

Previous co- or triculture studies of *D. mccartyi* strain 195 with corrinoid producers (e.g., *Acetobacterium woodii*, *Desulfovibrio desulfuricans*, and *Desulfovibrio vulgaris* Hildenborough, methanogens) demonstrated modest (i.e., up to 1-fold) increases of strain 195 cell numbers compared to pure culture growth yields (9, 24). These observations suggest that the other microbes provided some benefit to *Dehalococcoides*, and subsequent microarray studies found genes involved in cobamide salvage and lower ligand attachment downregulated in the co- or tri-cultures and in cultures amended with excess vitamin B_{12} (i.e., 100 μg liter^{-1}) (14, 24). Since all of these cocultures used medium amended with up to 100 μg of vitamin B_{12} liter^{-1}, the effects of the corrinoid producers on *Dehalococcoides* dechlorination activity remained speculative. The *Geobacter-Dehalococcoides* coculture studies conducted in vitamin B_{12}-free medium demonstrated *Dehalococcoides* requirement for cobalamin, which may have implications for bioremediation practice. PCE and TCE reductive dechlorination is carried out by different bacterial groups and generally precedes *Dehalococcoides* activity (5). *Geobacter lovleyi*, *Dehalobacter* sp., and likely other PCE-to-*cis*-DCE dechlorinators produce cobalamins, suggesting that an active PCE-dechlorinating community could provide *cis*-DCE- and VC-dechlorinating *Dehalococcoides* with the required cofactor. Hence, *Dehalococcoides* activity may be more robust at PCE/TCE-contaminated sites, whereas cobalamin limitations may occur at sites where dichloroethene(s) and VC are the major contaminants. To date, it has not been demonstrated that a lower bioremediation success rate at predominantly *cis*-DCE-contaminated sites is due to corrinoid cofactor limitations of the resident or augmented *Dehalococcoides* populations, but careful measurement of the cobamide pool could address this relevant question.

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