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Versatility in Corrinoid Salvaging and Remodeling Pathways Supports Corrinoid-Dependent Metabolism in *Dehalococcoides mccartyi*

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Corrinoids are cobalt-containing molecules that function as enzyme cofactors in a wide variety of organisms but are produced solely by a subset of prokaryotes. Specific corrinoids are identified by the structure of their axial ligands. The lower axial ligand of a corrinoid can be a benzimidazole, purine, or phenolic compound. Though it is known that many organisms obtain corrinoids from the environment, the variety of corrinoids that can serve as cofactors for any one organism is largely unstudied. Here, we examine the range of corrinoids that function as cofactors for corrinoid-dependent metabolism in *Dehalococcoides mccartyi* strain 195. *Dehalococcoides* bacteria play an important role in the bioremediation of chlorinated solvents in the environment because of their unique ability to convert the common groundwater contaminants perchloroethene and trichloroethene to the innocuous end product ethene. All isolated *D. mccartyi* strains require exogenous corrinoids such as vitamin B_{12} for growth. However, like many other corrinoid-dependent bacteria, none of the well-characterized *D. mccartyi* strains has been shown to be capable of synthesizing corrinoids *de novo*. In this study, we investigate the ability of *D. mccartyi* strain 195 to use specific corrinoids, as well as its ability to modify imported corrinoids to a functional form. We show that strain 195 can use only specific corrinoids containing benzimidazole lower ligands but is capable of remodeling other corrinoids by lower ligand replacement when provided a functional benzimidazole base. This study of corrinoid utilization and modification by *D. mccartyi* provides insight into the array of strategies that microorganisms employ in acquiring essential nutrients from the environment.

Corrinoids are essential cofactors for enzymes that facilitate carbon skeleton rearrangements, methyl group transfers, and reductive dehalogenation (4). Members of all three domains of life use corrinoid cofactors, yet the complete biosynthesis of corrinoids, which requires approximately 30 enzymatic steps, is performed only by a subset of prokaryotes (42, 52). The dependence of certain microbes on corrinoids produced by other organisms has previously been observed in ecosystems such as the mammalian gut, marine environments, and microbial consortia isolated from forest soil (9, 15, 24). A recent bioinformatic analysis revealed that while 76% of 540 sequenced bacterial genomes contain corrinoid-dependent enzymes, only 39% of these genomes contain the complete corrinoid biosynthesis pathway (55). This study also found that the *btuFCD* genes encoding a high-affinity corrinoid transporter are present in 76% of sequenced bacterial genomes, indicating that most bacteria are capable of taking up corrinoids from the environment (55). Based on these experimental and bioinformatic results, corrinoid cross-feeding is likely to be widespread in microbial communities.

Corrinoids are distinguished from one another in part based on the structure of the lower axial ligand, which can be a benzimidazole, purine, or phenolic compound (40) (Fig. 1). Corrinoid cofactors with different lower ligands (collectively termed cobamides) are not necessarily functionally equivalent as cofactors, as some cobamide-dependent metabolic processes have been shown to function only with a specific cobamide (5, 32). The best studied cobamide, cobalamin (also known as vitamin B_{12}), contains 5,6-dimethylbenzimidazole (DMB) as its lower ligand and is the only cobamide that is commercially available (Fig. 1A). In humans, cobalamin is clearly the preferred cobamide and may be the only corrinoid with significant biological activity. While a diverse range of corrinoids has been detected in microbial communities, very little is currently known about how microbes that import exogenous corrinoids respond to the presence of different corrinoids in the environment (1, 14; Y. J. Men, E. C. Seth, S. Yi, T. S. Crofts, R. H. Allen, M. E. Taga, and L. Alvarez-Cohen). The organohalide-respiring bacterium *Dehalococcoides mccartyi* strain 195 was chosen as a model organism for this study due to its obligate requirement for exogenously supplied corrinoids.

D. mccartyi plays a crucial role in the bioremediation of chlorinated solvents, as it is the only known organism capable of converting the common groundwater contaminants tetrachloroethene (PCE) and trichloroethene (TCE) to the nontoxic end product ethene (10, 20, 37, 48). *D. mccartyi* requires corrinoid cofactors for dechlorination and growth (19, 22, 23, 34, 36). Genome annotations of sequenced *D. mccartyi* isolates (strains 195, VS, BAVI, CBDB1, and GT) reveal the presence of three types of corrinoid-dependent enzymes (http://img.jgi.doe.gov/) (see Table S1 in the supplemental material) (29, 38, 45). Genes encoding reductive dehalogenases (RDases), which catalyze successive dehalogenation reactions in organohalide respiration, are present in 10 to 38 copies in each of the five published genome sequences, and evidence of corrinoid cofactor involvement in RDases has been shown experimentally in strains 195 and CBDB1 (23, 36).

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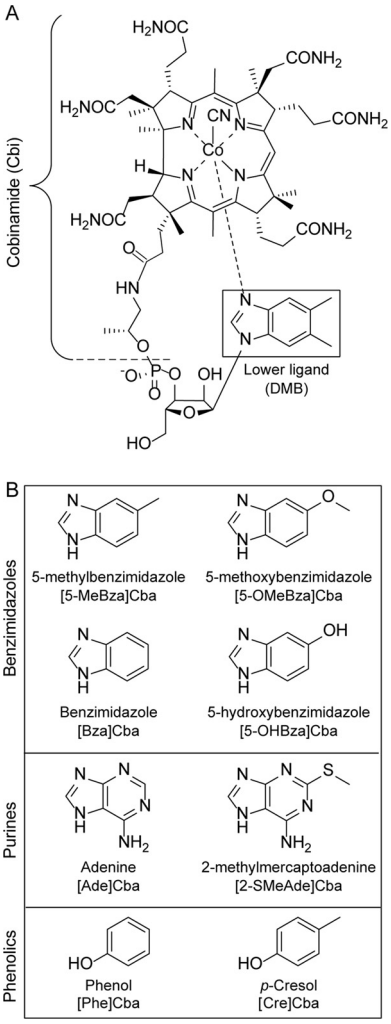

FIG 1 Structures of corrinoids and lower ligands. (A) Cyanocobalamin (vitamin B_{12}) is shown with the lower ligand, 5,6-dimethylbenzimidazole (DMB), boxed, and the structure of cobinamide (Cbi) is indicated by the bracket. (B) The structures of alternative lower ligands of corrinoids examined here are shown with the name of the lower ligand and the abbreviation for the corresponding complete cobamide (Cba) indicated below. A cobamide is defined as a corrinoid containing the nucleotide loop and a lower ligand.

The presence of 2 or 3 copies of the corrinoid-dependent ribonucleotide reductase (RNR) encoded by *nrdJ* in each strain indicates that corrinoid cofactors are also involved in DNA replication in *D. mccartyi* (see Table S1). In addition, homologs of the *acsCD* genes, predicted to encode a corrinoid iron sulfur protein (CFeSP) that facilitates methyl transfer reactions in an incomplete Wood-Ljungdahl acetyl-coenzyme A (CoA) pathway, are also present (http://img.jgi.doe.gov/) (29, 38, 45). In strain 195, transcripts and proteins corresponding to the RDase gene *tceA*, two of the three *nrdJ* paralogs, and both CFeSP genes have been detected (25, 26, 39).

Despite their dependence on corrinoid cofactors, portions of the corrinoid biosynthesis pathway are absent from the genomes of *D. mccartyi* strains, and all isolates require corrinoid supplementation when grown in pure culture. A possible exception was found in a recent metagenomic analysis of a TCE-dechlorinating enrichment culture in which putative genes for corrin ring synthesis from the precursor uroporphyrinogen III were identified in *D. mccartyi* strain ANAS2 (7). However, neither the ability to synthesize a corrinoid *de novo* nor the ability to grow without corrinoid supplementation has been shown experimentally in this strain.

D. mccartyi has been found in many different environments, including groundwater, subsurface soil, and river and marine sediments (21, 50). In these environments, *D. mccartyi* coexists with microbes that perform a variety of corrinoid-dependent metabolic functions such as the fermentation of short-chain organic compounds, acetogenesis, and methanogenesis (4, 12, 35, 41). Microorganisms carrying out these processes have been found to produce different corrinoids with benzimidazolyl, purinyl, and phenolyl lower ligands (40, 46, 47) (Fig. 1). As *D. mccartyi* is likely to encounter multiple corrinoids in the environment, it is important to understand which corrinoids can support its growth.

The genomes of all sequenced *D. mccartyi* strains contain putative corrinoid salvaging and remodeling genes, which may help fulfill the requirement for exogenous corrinoids (http://img.jgi.doe.gov/) (29, 38, 45). Corrinoid salvaging involves the uptake and modification of an incomplete corrinoid such as cobinamide (Cbi; a corrinoid lacking a lower ligand) to form a cobamide through the attachment of an upper ligand, nucleotide loop, and lower ligand (Fig. 2) (13). The enzyme CbiZ has been shown in *Rhodobacter sphaeroides* to catalyze the removal of the lower ligand and nucleotide loop of adeninylcobamide ([Ade]Cba), a corrinoid that *R. sphaeroides* can import from the environment but is incapable of using as a cofactor without modification (16, 17). The process of removing and replacing a lower ligand is termed cobamide remodeling. Corrinoid salvaging and remodeling activities have not yet been studied in *D. mccartyi* or any other organism that requires exogenous corrinoids.

Here, we demonstrate that the range of corrinoids that support dechlorination and growth of *D. mccartyi* strain 195 is restricted to just three structurally related cobamides. In addition, we find that strain 195 is capable of salvaging and remodeling a variety of nonfunctional corrinoids to form cobamides that support dechlorination and growth, as long as a benzimidazole lower ligand base is also provided. Thus, while strain 195 is incapable of producing a corrinoid or lower ligand *de novo*, it can assemble functional cobamides from cobamide precursors or from cobamides that do not function as cofactors. These activities may enable *D. mccartyi* to carry out corrinoid-dependent metabolism in microbial communities in which structurally diverse corrinoids are present.

MATERIALS AND METHODS

Identification of corrinoid salvaging and remodeling genes in *D. mccartyi* strains. The amino acid sequences corresponding to experimentally characterized genes involved in corrinoid salvaging and remodeling pathways in three microorganisms, *Salmonella enterica* serovar Typhimurium, *Escherichia coli* K-12, and *Methanocaldococcus jannaschii*, were compared to sequences in each of the *D. mccartyi* genomes using reciprocal BLAST analysis (see Table S2 in the supplemental material). In the first step, each characterized protein sequence was queried against each *D. mccartyi* genome to identify the highest scoring orthologs using BLASTP (2). In the second step, reciprocal BLASTP searches were conducted using *D. mccartyi* ortholog sequences to query against the genome containing the characterized protein of interest. An Expect (E) value of $<10^{-17}$, query coverage of at least 48%, and a minimum of 24% sequence identity were used as stringency thresholds for determining a valid best hit.

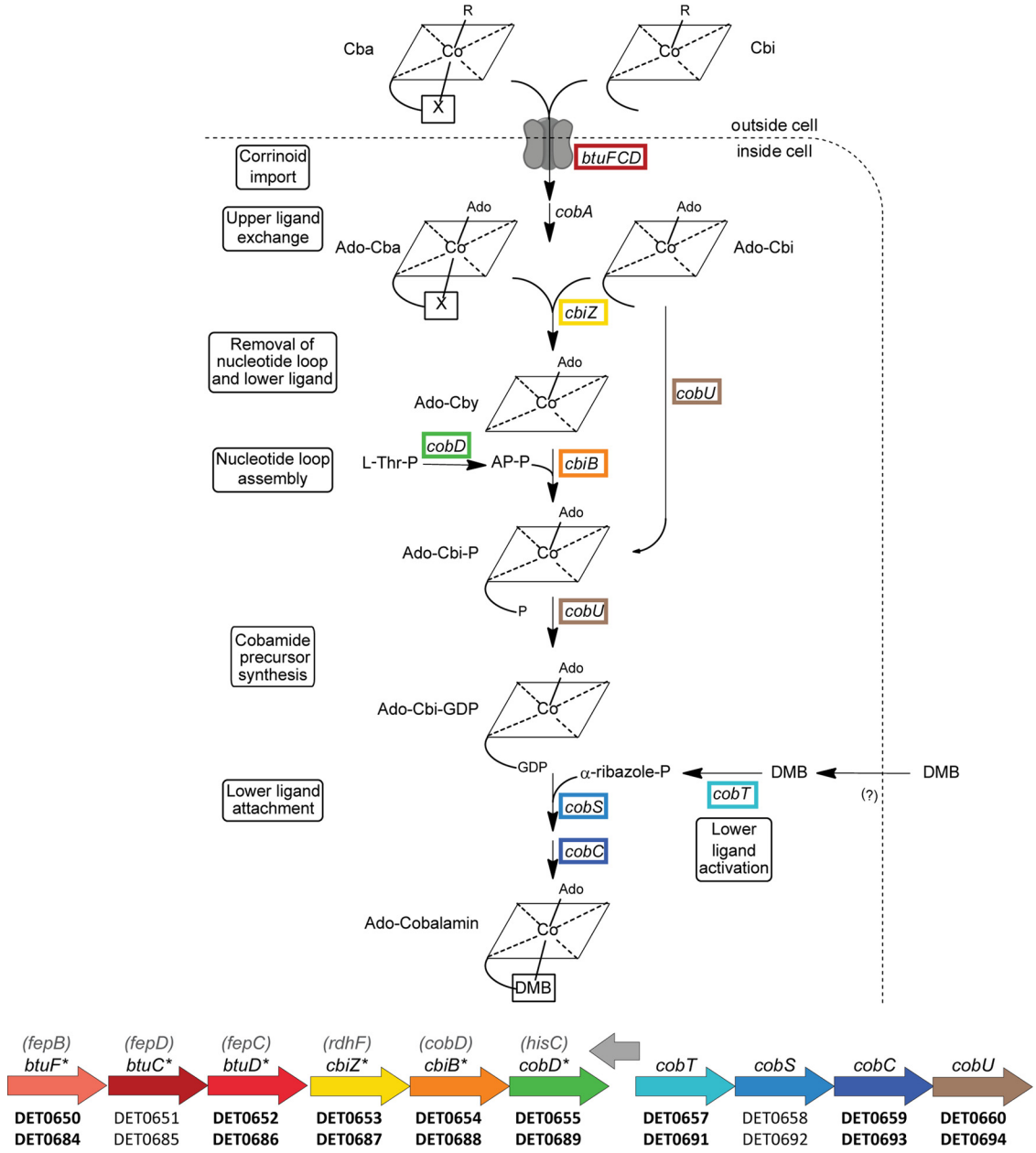

FIG 2 Model of corrinoid salvaging and remodeling pathways in *D. mccartyi*. Extracellular Cba and Cbi are imported by a high-affinity corrinoid transporter. Within the cell, the upper ligand is exchanged with deoxyadenosine (Ado). Salvaging of cobinamide occurs through one of two redundant pathways encoded by either *cbiZ* and *cbiB* or *cobU*. Remodeling of cobamides begins with the removal of the lower ligand (X) and the nucleotide loop. Cobamides are formed from the precursor Ado-Cbi-GDP and an activated lower ligand. Putative homologs of genes involved in each step of the pathway that are encoded by *D. mccartyi* genomes are shown. Most genes in the pathway are present in a single genomic locus shown at the bottom, with the locus numbers in strain 195 shown below each gene. Locus numbers shown in bold indicate the genes that are expressed in both transcripts and proteins (25, 26, 39). Arrows represent the direction of each gene. The gray arrow indicates a conserved hypothetical open reading frame. Asterisks correspond to new annotations proposed here. Gene names in parentheses indicate the previous annotation. Ado, adenosyl; Cby, cobyric acid; P, phosphate; AP, aminopropanol.

Extraction and purification of cobamides. [Bza]Cba, [5-MeBza]Cba, [2-SMeAde]Cba, and [Cre]Cba (see Fig. 1 for abbreviation descriptions) were extracted from bacterial cultures and purified as described previously (1). [Ade]Cba was extracted from 6 liters of *S. enterica* serovar Typhimurium strain LT2 grown aerobically for 48 h at 37°C in no-carbon medium E (NCE) containing 10 mM glycerol, 80 mM 1,2-propanediol, and 1 μM Cbi as described previously (16). [5-OHBza]Cba was extracted from 4 liters of *Methanosarcina barkeri* strain Fusaro grown anaerobically for 14 days at 34°C in defined mineral salt medium containing a N_2-CO_2 headspace, 0.5% (vol/vol) methanol, and a modified Wolin vitamin solution without cobalamin (19). Cells were harvested by centrifugation (8,000 × *g*, 4°C, 20 min). Cell pellets were resuspended in 20 ml of methanol with 20 mg potassium cyanide per gram (wet weight) of cells and incubated at 60°C for 1.5 h with vortex mixing every 20 min. Cellular debris was removed by centrifugation at 40,000 × *g* for 1 h, and the supernatant was dried in a rotary evaporator. Samples were resuspended in 10 to 20 ml deionized water, desalted with a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA), and eluted in 2 ml methanol. The elu-

ates were dried overnight in a vacuum desiccator, resuspended in deionized water, and stored at −80°C.

High-performance liquid chromatography (HPLC) purification was performed with an Agilent Series 1200 system (Agilent Technologies, Santa Clara, CA) equipped with a diode array detector. Samples were injected onto an Agilent Eclipse plus C_{18} column (5 μm, 9.4 by 250 mm) at 45°C, with a flow rate of 1.8 ml min^{-1}. Samples were separated using a solvent system consisting of solvent A, 0.1% formic acid in water, and solvent B, 0.1% formic acid in methanol, with a linear gradient of 10 to 40% solvent B over 17 min. Fractions were collected using an Agilent 1200 series fraction collector and dried under a vacuum. The concentrations of cobamides were measured spectrophotometrically at 367.5 nm in deionized water. A molar extinction coefficient of 30,800 mol^{-1} cm^{-1} was used for quantification (1). The identity of each corrinoid was confirmed by mass spectrometry.

***D. mccartyi* strain 195 culture conditions.** *D. mccartyi* strain 195 (37) was grown anaerobically in a defined mineral salt medium containing 7 μl TCE and 2 mM sodium acetate as the electron acceptor and carbon source, respectively (49). Hydrogen was provided as the electron donor in a headspace of H_2-CO_2 (80:20 [vol/vol]). The medium was amended with a modified Wolin vitamin solution excluding cobalamin. When indicated, 36.7 nM cobalamin, an alternate purified corrinoid, Cbi, and/or the lower ligand base Cre, 5-OHBza, Bza, 5-OMeBza, 5-MeBza, or DMB were added to the culture (19). The cultures amended with different corrinoids were incubated for 7 to 14 days until the TCE was depleted from the culture provided with cobalamin.

Extraction and analysis of corrinoids from *D. mccartyi* 195. Three to six 100-ml cultures of strain 195 were harvested by centrifugation at 15,000 × *g*, 4°C for 15 min after 18 to 21 days of growth, when 95 to 98% of two sequential doses of 7 μl TCE were depleted. The cell pellets were then resuspended in methanol and stored at −20°C until extraction. The culture supernatants were individually applied to C_{18} Sep-Pak cartridges (Waters Associates, Milford, MA), washed with 50 ml water, and eluted with 3 ml methanol. Corrinoid extractions from cell pellets and supernatants were performed as described above. Corrinoid concentrations reported represent the amount present in the cell pellet and supernatant combined.

Analytical methods. Chloroethenes and ethene in the culture headspace were measured by gas chromatography (Agilent 7890A; Agilent technologies, Palo Alto, CA) with a 30-m J&W capillary column and a flame ionization detector as described previously (19, 49). Cl^- released was calculated from metabolic dechlorination from TCE to vinyl chloride (VC) as described previously (11). Cell number in the cultures was determined by quantitative real-time PCR with primers specific to the *D. mccartyi* strain 195 *tceA* gene, using a StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA) as described previously (56). Dechlorination and cell yield are reported as the percentage of the average level observed in cultures containing cobalamin.

Corrinoid extracts from strain 195 were analyzed with an Agilent Technologies 6410 liquid chromatograph-triple quadrupole mass spectrometer (Y. J. Men, E. C. Seth, S. Yi, T. S. Crofts, R. H. Allen, M. E. Taga, and L. Alvarez-Cohen, unpublished). Briefly, samples were injected onto a ZORBAX Eclipse Plus C_{18} column (1.8 μm, 3.0 by 50 mm) (Agilent Technologies, Santa Clara, CA) using an Agilent 1200 series autosampler. The flow rate was 0.5 ml min^{-1}, and elution solvents consisted of 0.1% formic acid in water (solvent A) and in methanol (solvent B). Samples were eluted with a solvent program that was started at 18% solvent B, increased to 21% solvent B in a linear gradient over 3 min, and remained at 21% solvent B for 2 min. The tandem triple quadrupole mass spectrometry was set for multiple reaction monitoring for quantification of each corrinoid except [Cre]Cba in positive electrospray ionization mode with the fragmentor voltage set to 135 V and the collision energy set to 45 V. The limit of quantification for this method was 5 nM. [Cre]Cba was quantified using selective ion mode (SIM) with a limit of quantification of 0.2 μM.

RESULTS

Bioinformatic analysis of corrinoid salvaging and remodeling genes in sequenced *D. mccartyi* genomes. A genomic locus containing 11 genes, five of which are annotated as functioning in corrinoid salvaging and remodeling, is present in at least one copy in each of the five fully sequenced *D. mccartyi* genomes (Fig. 2). In each *D. mccartyi* genome, homologs of these genes are arranged in the same orientation and order. To identify additional genes that may be involved in corrinoid salvaging and remodeling, reciprocal BLAST analysis of genes at this locus was performed against the genomes of *S. enterica* serovar Typhimurium, *E. coli* K-12, and *M. jannaschii*, three organisms for which experimental evidence for the functions of genes involved in corrinoid salvaging or remodeling is available (6, 8, 18, 33, 54). This analysis indicated that 10 of the 11 genes at this locus are likely to be involved in these processes (Fig. 2; see also Table S2 in the supplemental material). Our model for how corrinoid salvaging and remodeling might occur in *D. mccartyi* is shown in Fig. 2.

The gene *hisC*, previously annotated as encoding L-histidinol phosphate aminotransferase, was identified as being more similar to *cobD*, which encodes an enzyme that decarboxylates L-Thr-P to yield AP-P in the anaerobic corrinoid biosynthesis pathway (Fig. 2; see also Table S2 in the supplemental material) (8). Furthermore, the gene previously annotated as *cobD* was reannotated as *cbiB*, which encodes an enzyme that catalyzes the conversion of AP-P and Ado-Cby to Ado-Cbi-P (43) (Fig. 2). Another gene at this locus, *cbiZ*, was found to be misannotated in two of the *D. mccartyi* strains as *rdhF*. Potential homologs of *cbiZ* are also present in 2 to 7 copies in other regions of the *D. mccartyi* genomes (see Table S2).

Reciprocal best BLAST hit analysis also suggests that three genes in the locus that are annotated as *fepBDC* are more likely to encode the ABC transporter *btuFCD*, which is involved in importing corrinoids from the environment. While *fepB* was found to have the highest sequence similarity to *btuF*, which encodes the periplasmic corrinoid-binding protein, *fepD* and *fepC* are more similar to siderophore transporter components. Although ABC transporters for corrinoids, siderophores, and other substrates are difficult to distinguish from one another based on sequence alone, the presence of these genes adjacent to other corrinoid salvaging genes suggests that they function in corrinoid transport. Furthermore, a previous transcriptional study showed that expression of these genes was induced under cobalamin-limited conditions (26). Additionally, the existence of a high-affinity corrinoid transporter in *D. mccartyi* is supported by their ability to carry out growth and TCE dechlorination in the presence of as little as 0.8 nM cobalamin (26). In strain 195, previous gene expression and proteomic analyses demonstrated that 8 of the 11 genes at this locus are expressed in both transcripts and proteins (Fig. 2) (25, 26, 39).

Specific corrinoid requirements of strain 195. Although *D. mccartyi* isolates are routinely supplied with cobalamin to fulfill their corrinoid requirements, it is possible that they are capable of using other corrinoids as well. Because cobamides other than cobalamin are not commercially available, we purified six additional cobamides from bacterial cultures by guided biosynthesis as described in Materials and Methods. Parallel cultures of strain 195 were provided with one of seven different corrinoids, and TCE dechlorination activity and cell growth of these cultures were

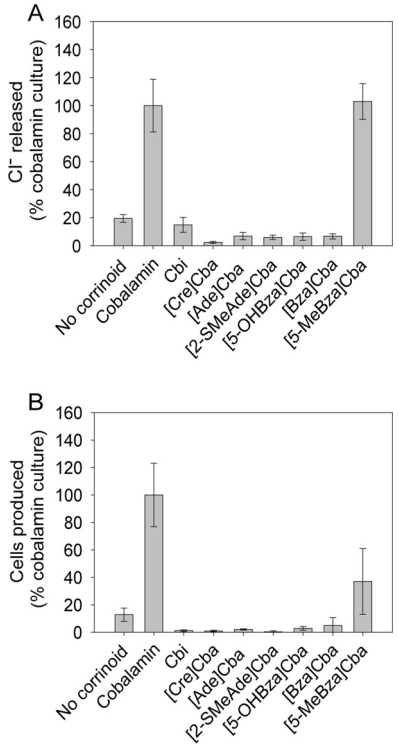

FIG 3 Dechlorination and growth of *D. mccartyi* strain 195 in the presence of different corrinoids. Cl^- released from TCE dechlorination (A) and cell number are shown for cultures grown in the absence or presence of each of eight corrinoids (B). The data shown are the mean values from three independent experiments with error bars representing one standard deviation.

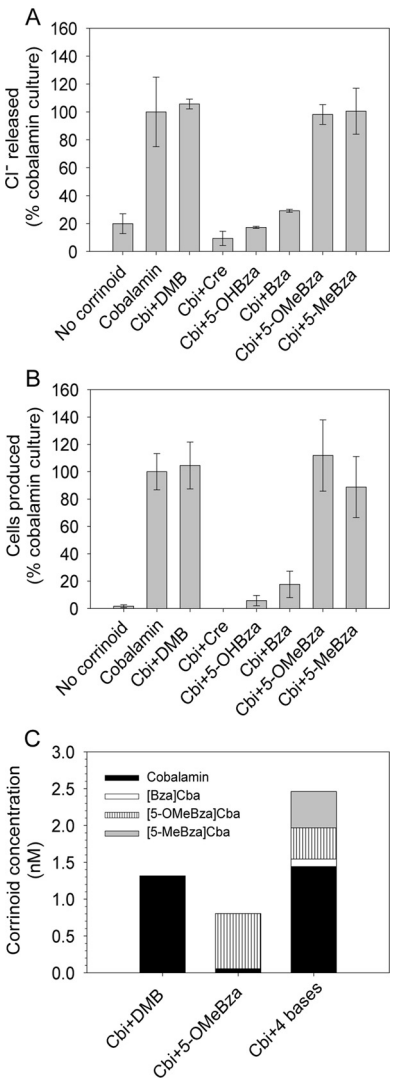

FIG 4 Dechlorination and growth of *D. mccartyi* strain 195 in the presence of Cbi plus different lower ligand bases. Cl^- released from TCE dechlorination (A) and cell number are shown for cultures grown in the absence and presence of Cbi plus each of six different lower ligand bases (B). (C) Quantification of corrinoids extracted from cultures provided with Cbi plus DMB, 5-OMeBza, or a mixture of Bza, 5-MeBza, 5-OMeBza, and DMB in equal molar concentrations (Cbi + 4 bases) is shown. The culture grown with Cbi plus 5-OMeBza contains 0.05 nM cobalamin that was present in the inoculum.

compared to those of cultures supplied with cobalamin. The corrinoids tested included cobamides with phenolyl, purinyl, and benzimidazolyl lower ligands, as well as Cbi. Our results showed that only two of the benzimidazolyl cobamides, cobalamin and [5-MeBza]Cba, were capable of supporting dechlorination of TCE and cell growth, while the other corrinoids tested were not (Fig. 3). Dechlorination activity of cultures grown with [5-MeBza]Cba was comparable to cultures with cobalamin (Fig. 3A). While fewer cells were produced in cultures with [5-MeBza]Cba added than in cobalamin-supplied cultures, cell growth was enhanced relative to cultures supplied with any other corrinoid (Fig. 3B). Although sequence analysis suggests that strain 195 possesses redundant pathways for Cbi salvaging (Fig. 2) (45), the addition of Cbi alone failed to support dechlorination or cell growth (Fig. 3).

Cbi salvaging and lower ligand attachment in strain 195. In order to investigate whether the Cbi salvaging pathways are functional in strain 195, we provided cultures with both Cbi and DMB, the lower ligand of cobalamin (Fig. 1A). We reasoned that the failure to use Cbi alone could be due to an inability to synthesize a lower ligand base such as DMB or 5-MeBza to form a functional cobamide. Our results show that cultures provided with both Cbi and DMB exhibited TCE dechlorination and cell growth similar to those of cultures with cobalamin added (Fig. 4A and B). Moreover, cobalamin was detected in corrinoid extracts from these cultures (Fig. 4C), which suggests that strain 195 is capable of salvaging Cbi, synthesizing the nucleotide loop, and activating and attaching DMB (Fig. 2).

Since we observed that the addition of Cbi plus DMB supports TCE dechlorination and growth, we next investigated whether other lower ligand bases could substitute for DMB. We provided parallel cultures of strain 195 with Cbi plus one of five lower ligand bases and measured dechlorination and growth. In cultures provided with Cbi and either Cre or 5-OHBza, dechlorination and growth did not exceed background levels (Fig. 4A and B). These results confirm those seen when cobamides containing Cre or 5-OHBza were provided to strain 195. Cre appeared to be toxic, as the cell number in these cultures decreased after incubation. A culture provided with Cbi and Bza showed a small amount of dechlorination and growth, though this effect was not observed when the complete corrinoid [Bza]Cba was provided (Fig. 3 and 4). In contrast, the addition of Cbi and either 5-OMeBza or 5-MeBza resulted in levels of TCE dechlorination and growth indis-

tinguishable from those of cultures grown with cobalamin or Cbi plus DMB (Fig. 4A and B). When corrinoids extracted from cultures provided with Cbi and 5-OMeBza were analyzed, [5-OMeBza]Cba was detected (Fig. 4C).

We also investigated whether *D. mccartyi* shows preferential attachment of particular lower ligands when four benzimidazoles, DMB, 5-OMeBza 5-MeBza, and Bza, were provided in equal concentrations together with Cbi. We observed higher levels of attachment of DMB, as cobalamin represented 59% of the total extracted corrinoids, followed by nearly equal levels of [5-MeBza]Cba and [5-OMeBza]Cba at 20 and 17%, respectively (Fig. 4C). [Bza]Cba, which did not fully support dechlorination, was detected in only 4% of the total extracted corrinoids. This suggests either that these lower ligand bases are not taken up in equal amounts or that lower ligand activation and attachment enzymes act most efficiently on benzimidazoles that support dechlorination and growth.

Cobamide remodeling. Our bioinformatic analysis of *D. mccartyi* genomes suggests that the complete cobamide remodeling pathway is present (Fig. 2). Although we found that some cobamides do not support TCE dechlorination or growth (Fig. 3), we reasoned that cobamide remodeling did not occur because a functional lower ligand was not provided. When DMB was provided in addition to any of the nonfunctional cobamides, dechlorination was restored to levels observed when cobalamin alone was added (Fig. 5A). Growth in these cultures was significantly improved relative to that in cultures with these cobamides provided alone (compare Fig. 3B and 5B).

The restoration of growth and dechlorination activity in cultures provided with a nonfunctional cobamide plus DMB suggests that the nonfunctional cobamide is converted to cobalamin, presumably through the activities of the corrinoid salvaging and remodeling pathway genes (Fig. 2). Consistent with this hypothesis, cobalamin was detected in corrinoid extracts from these cultures at levels exceeding 0.8 nM, the minimum concentration required for growth (Fig. 5C) (26). Although all nonfunctional cobamides were remodeled when DMB was provided, the efficiency of this process appeared to depend on the particular cobamide.

Replacement of the lower ligand with DMB also occurred when [5-MeBza]Cba, a cobamide capable of supporting dechlorination and growth, was provided (Fig. 5C). Similarly, when cultures were provided with cobalamin plus 5-MeBza, [5-MeBza]Cba was detected in the corrinoid extract (Fig. 5C). Together, these results demonstrate that *D. mccartyi* is capable of modifying both functional and nonfunctional corrinoids.

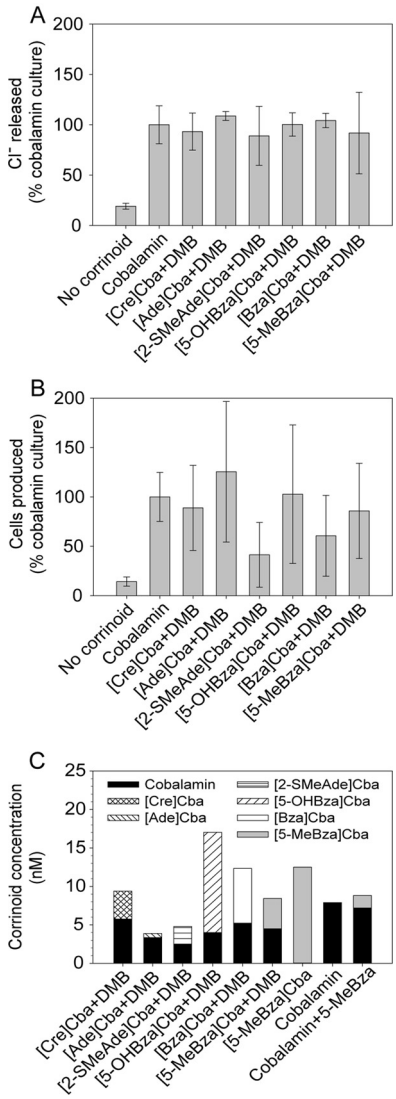

FIG 5 Modification of cobamides by *D. mccartyi* strain 195 grown in the presence of DMB. Cl^- released from TCE dechlorination (A) and cell number are shown for cultures grown with each of six cobamides plus DMB (B). (C) Quantification of corrinoids extracted from the six cultures containing cobamide plus DMB are shown. Corrinoids present in cultures grown with [5-MeBza]Cba plus DMB and with cobalamin plus 5-MeBza are also shown.

DISCUSSION

Many microbes depend on other organisms in their environment for the biosynthesis of essential metabolites, including corrinoids. Given the diversity of corrinoid structures, organisms that require exogenous corrinoids must have mechanisms to acquire the specific corrinoids that function as cofactors for their corrinoid-dependent enzymes. In mammals, the cobalamin absorption pathway effectively prevents nonfunctional corrinoids obtained from food from entering cells through a series of highly specific protein-ligand interactions (27). However, in microbes, the range of corrinoids that function as cofactors and the mechanisms by which functional corrinoids are obtained were previously less clear. In this study, we demonstrate that *D. mccartyi* strain 195 can use only three benzimidazolyl cobamides efficiently as cofactors but can make use of a broader range of corrinoids through its corrinoid salvaging and remodeling activities. The inability to synthesize corrinoids *de novo* despite the presence of corrinoid-dependent enzymes is common among bacteria (55) and highlights the importance of corrinoid production by the microbial community.

This analysis of the corrinoid requirements of strain 195 demonstrates that the structure of the lower ligand base of a corrinoid affects its ability to function as a cofactor in dechlorination and other processes essential for cell growth. We found that strain 195 is surprisingly restrictive in its corrinoid requirements, as only a subset of the benzimidazolyl cobamides function efficiently as cofactors, in contrast to *S. enterica* or the corrinoid bioassay strain *Lactobacillus leichmannii* which can use both benzimidazolyl and purinyl cobamides (3, 53). The requirement for benzimidazolyl cobamides for cell growth may be due to the ability of these cob-

amides to modulate the reactivity of the upper ligand in enzymes that utilize cobamides with the lower ligand coordinated to the cobalt center (i.e., "base-on"). DNA synthesis in *D. mccartyi* is predicted to be dependent on the class II RNR NrdJ, an enzyme that functions with the cobamide bound in the base-on form (31). In contrast, RDases and CFeSPs, the two other types of corrinoid-dependent enzymes present in *D. mccartyi*, have been shown in other organisms to function with the corrinoid cofactor bound in the "base-off" position (30, 44, 51). The requirement of *D. mccartyi* for benzimidazolyl cobamides differs from that of *Sulfurospirillum multivorans*, which uses the purinyl cobamide noradeninylcobamide as a cofactor for its PCE RDase, and is capable of reductive dechlorination of PCE only to dichloroethene (28). This could be due to differences in the catalytic mechanisms of the RDases or adaptations of the enzymes to the corrinoids available in their respective environments.

Our results also show that strain 195 can produce functional corrinoids by attaching DMB, 5-MeBza, or 5-OMeBza (and to a lesser extent, Bza) to Cbi. This activity is likely enabled by the combined functions of several enzymes present in all sequenced *D. mccartyi* genomes that are predicted to perform each of the steps necessary for the conversion of Cbi to a cobamide (Fig. 2). Our finding that the addition of Cbi plus 5-OHBza does not support TCE dechlorination or growth indicates that *D. mccartyi* lacks the ability to transform 5-OHBza into DMB, an activity that has been described in *Eubacterium limosum* (40). The production of different levels of each corrinoid when four benzimidazole bases were supplied together suggests that preferential attachment of specific lower ligands occurs even within a single structural class of lower ligands. Selectivity in the activation or attachment of particular lower ligands could play an important role in ensuring that functional cobamides are produced when multiple potential lower ligands are present in the environment. We are currently examining the lower ligand attachment specificity of the CobT enzyme of strain 195 *in vitro* to determine whether substrate specificity in CobT influences the attachment efficiency of different benzimidazoles.

The potential of *D. mccartyi* to make use of a variety of corrinoids is demonstrated by its ability to convert nonfunctional cobamides to cobalamin when DMB is also available. Interestingly, we found that strain 195 also remodels two functional corrinoids, cobalamin and [5-MeBza]Cba. This suggests that all corrinoids imported by strain 195 are subject to modification regardless of their ability to function as cofactors. This is in contrast to the remodeling activity observed in *R. sphaeroides* (16, 17), in which the CbiZ enzyme does not act on cobalamin. Remodeling of cobalamin, presumably by the action of members of the gut microbiota, has also been observed in the human gut. A recent study shows that dietary supplementation with cobalamin results in an increase in the concentration of Cbi and five other cobamides, suggesting that cobalamin is converted to a variety of other corrinoids (1).

The ability of *D. mccartyi* to salvage and remodel corrinoids only when free DMB is provided underscores the importance of the availability of functional lower ligands. While much previous attention has been given to organisms that synthesize corrinoids, the identification of organisms that synthesize free lower ligand bases now appears to be of equal importance. Identification of the genes involved in the anaerobic biosynthesis of benzimidazoles would broaden our understanding of the organisms that contribute to lower ligand production in microbial communities.

The corrinoid salvaging and remodeling abilities of *D. mccartyi* demonstrate how a microbe can rely on corrinoid-dependent metabolism without the need to produce a large cofactor *de novo*. Bioinformatic analyses have shown that many other microbes lack the complete corrinoid biosynthetic pathway though they possess corrinoid-dependent enzymes. Strategies used by *D. mccartyi* to acquire functional corrinoids may be common in microbial communities. Furthermore, this analysis of corrinoid acquisition by *D. mccartyi* brings us closer to understanding the means by which microbes obtain nutrients from other organisms in environmental communities.

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