Incomplete Wood–Ljungdahl pathway facilitates one-carbon metabolism in organohalide-respiring *Dehalococcoides mccartyi*

Wei-Qin Zhuang[a,1], Shan Yi[a,1], Markus Bill[b], Vanessa L. Brisson[a], Xueyang Feng[c], Yujie Men[a], Mark E. Conrad[b], Yinjie J. Tang[c], and Lisa Alvarez-Cohen[a,b,2]

[a]Department of Civil and Environmental Engineering, University of California, Berkeley, CA 94720-1710; [b]Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; and [c]Energy, Environmental and Chemical Engineering, Washington University, St. Louis, MO 63130

Edited by James M. Tiedje, Michigan State University, East Lansing, MI, and approved March 13, 2014 (received for review November 16, 2013)

The acetyl-CoA "Wood–Ljungdahl" pathway couples the folate-mediated one-carbon (C1) metabolism to either CO_2 reduction or acetate oxidation via acetyl-CoA. This pathway is distributed in diverse anaerobes and is used for both energy conservation and assimilation of C1 compounds. Genome annotations for all sequenced strains of *Dehalococcoides mccartyi*, an important bacterium involved in the bioremediation of chlorinated solvents, reveal homologous genes encoding an incomplete Wood–Ljungdahl pathway. Because this pathway lacks key enzymes for both C1 metabolism and CO_2 reduction, its cellular functions remain elusive. Here we used *D. mccartyi* strain 195 as a model organism to investigate the metabolic function of this pathway and its impacts on the growth of strain 195. Surprisingly, this pathway cleaves acetyl-CoA to donate a methyl group for production of methyl-tetrahydrofolate (CH_3-THF) for methionine biosynthesis, representing an unconventional strategy for generating CH_3-THF in organisms without methylene-tetrahydrofolate reductase. Carbon monoxide (CO) was found to accumulate as an obligate by-product from the acetyl-CoA cleavage because of the lack of a CO dehydrogenase in strain 195. CO accumulation inhibits the sustainable growth and dechlorination of strain 195 maintained in pure cultures, but can be prevented by CO-metabolizing anaerobes that coexist with *D. mccartyi*, resulting in an unusual syntrophic association. We also found that this pathway incorporates exogenous formate to support serine biosynthesis. This study of the incomplete Wood–Ljungdahl pathway in *D. mccartyi* indicates a unique bacterial C1 metabolism that is critical for *D. mccartyi* growth and interactions in dechlorinating communities and may play a role in other anaerobic communities.

reductive dechlorination | ^{13}C isotope analysis | acetyl-CoA synthase

The acetyl-CoA "Wood–Ljungdahl" pathway consists of two joined linear branches that couple folate-mediated one-carbon (C1) metabolism to CO_2 reduction or acetate oxidation via acetyl-CoA (Fig. 1). This pathway plays crucial roles in both microbial energy conservation and carbon assimilation under anaerobic conditions (1–3). Initially elucidated in homoacetogenic bacteria operating in a reductive direction, this pathway is now known to exist in a variety of forms that are used in reductive or oxidative directions in bacteria and archaea with diverse respiratory processes, including methanogenesis, hydrogen generation, sulfate reduction, and possibly anaerobic ammonium oxidation (4–9). An incomplete Wood–Ljungdahl pathway was identified in genome annotations of all five sequenced strains of the organohalide-respiring bacterium *Dehalococcoides mccartyi* (Fig. 1) [strain195, VS, BAV1, CBDB1 (10–12), and GT (http://img.jgi.doe.gov)]. *D. mccartyi* strains play a crucial role in the bioremediation of chlorinated solvents, as they are the only known organisms capable of converting the common groundwater contaminants tetrachloroethene and trichloroethene (TCE) to the nontoxic end product ethene (13). However, the cellular functionality and ecological impact of the incomplete Wood–Ljungdahl pathway of *D. mccartyi* are currently unknown.

In annotations of the incomplete Wood–Ljungdahl pathway, four essential gene homologs appear to be missing (10–12) (http://img.jgi.doe.gov). One of the missing genes, *metF*, corresponds to methylene-tetrahydrofolate reductase (MTHFR) in the C1 metabolism pathway, which reduces 5-, 10-methylene-tetrahydrofolate (CH_2-THF) to 5-methyl-tetrahydrofolate (CH_3-THF), a required methyl donor for methionine biosynthesis (14). Although deletion of *metF* in bacteria often leads to methionine auxotrophy, *D. mccartyi* exhibits the ability of de novo methionine biosynthesis without this gene, suggesting the existence of an alternate mechanism for generation of the methyl donor for methionine biosynthesis (15–18).

The other three missing gene homologs, *acsE*, *fdh*, and *acsA*, correspond to a methyltransferase (MeTr), formate dehydrogenase (FDH), and the carbon monoxide dehydrogenase (CODH) subunit of a bifunctional enzyme-complex CODH/acetyl-CoA synthase (ACS), respectively. When functioning in the reductive direction, MeTr is responsible for methyl transfer from CH_3-THF to the corrinoid iron–sulfur protein (CFeSP) for synthesizing acetyl-CoA, and FDH and CODH reduce CO_2 to formate and carbon monoxide (CO), respectively (Fig. 1). Indeed, *D. mccartyi* strains 195 and CBDB1 have been found to be incapable of reducing CO_2 to either the methyl or carbonyl group of acetyl-CoA in ^{13}C-labeled tracer experiments (16, 18). However, as the reactions catalyzed by MeTr, FDH, and CODH are often

Significance

We have studied the functionality of an incomplete acetyl-CoA "Wood–Ljungdahl" pathway in a strictly organohalide-respiring bacterium, *Dehalococcoides mccartyi*. We found that in addition to its ability to incorporate exogenous formate, this pathway cleaves acetyl-CoA to generate methyl-tetrahydrofolate for methionine biosynthesis, serving as a unique substitute of the missing methylene-tetrahydrofolate reductase function. We also found that accumulation of carbon monoxide (CO), an obligate by-product from acetyl-CoA cleavage, inhibits *D. mccartyi* axenic cultures, but can be ameliorated by the presence of a CO-oxidizing organism, resulting in an unusual syntrophic association. The understanding of the products and biosynthetic functions of this incomplete Wood–Ljungdahl pathway improves our knowledge of alternate central metabolic strategies used by environmental microorganisms.

Author contributions: W.-Q.Z., S.Y., Y.J.T., and L.A.-C. designed research; W.-Q.Z., S.Y., M.B., V.L.B., X.F., and Y.M. performed research; M.E.C. and Y.J.T. contributed new reagents/analytic tools; W.-Q.Z., S.Y., M.B., V.L.B., X.F., Y.M., Y.J.T., and L.A.-C. analyzed data; and W.-Q.Z., S.Y., M.B., V.L.B., Y.J.T., and L.A.-C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]W.-Q.Z. and S.Y. contributed equally to this work.

[2]To whom correspondence should be addressed. E-mail: alvarez@ce.berkeley.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1321542111/-/DCSupplemental.

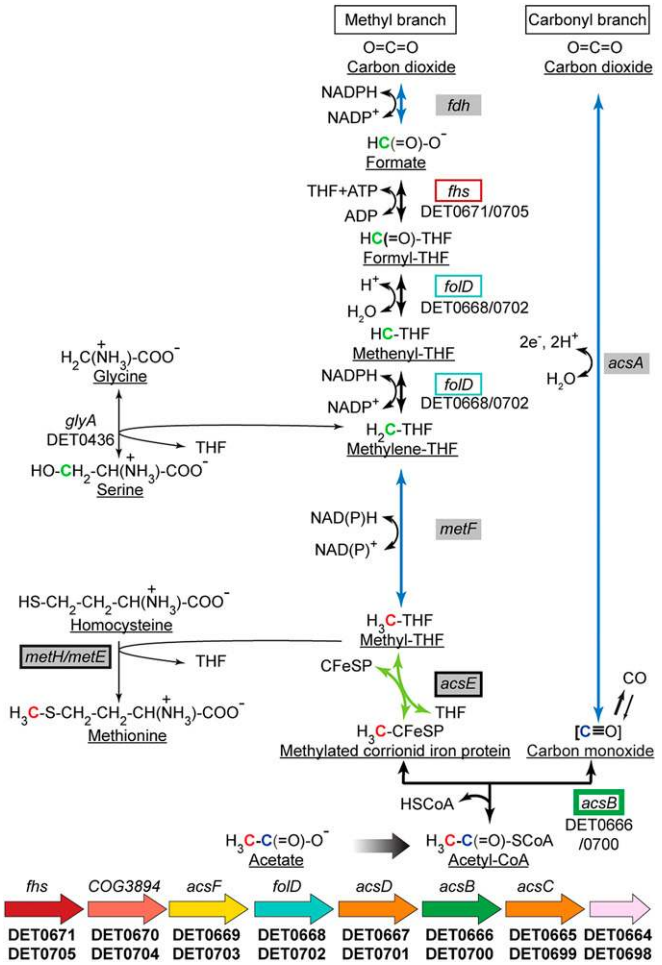

Fig. 1. Schematic of the Wood–Ljungdahl pathway (thick arrows) with associated steps of serine and methionine biosynthesis pathways (thin arrows). Colored arrows indicate the reactions missing in the genome annotations of all five sequenced *D. mccartyi* isolates (blue, confirmed missing functions; light green, detected function in this study). Gray arrow indicates that acetate fuels central carbon metabolism via acetyl-CoA. Different from the conventional Wood–Ljungdahl pathway where CO is a transient intermediate often channeled to downstream enzymes to prevent leakage (shown in brackets) (1–3), the incomplete pathway in *D. mccartyi* strain 195 produces or incorporates free CO. Three-color–labeled carbons indicate three labeling experiments in strain 195 (see *Results* for details): green for [^{13}C]formate, red for [2-^{13}C]acetate, blue for [1-^{13}C]acetate. Putative homologs of genes involved in each step of the pathway in the strain 195 genome are present in a single genomic locus shown at the bottom with locus numbers (12). The pink arrow indicates a hypothetical ORF. Colored boxes designate the identified homologs. Gray-shaded boxes indicate missing homologs in the genome annotation of strain 195. Framed gray-shaded boxes indicate the detected functions without annotated homologs [*metH*/*metE* (16, 18); *acsE*, see *Results* and *Discussion* for details]. Gene abbreviations: *acsA*, carbon monoxide dehydrogenase (CODH); *acsB*, acetyl-CoA synthase (ACS); *acsCD*, corrinoid iron sulfur protein (CFeSP); *acsE*, methyltransferase (MeTr); *acsF*, ACS chaperone; COG3894, ferredoxin; *fdh*, formate dehydrogenase (FDH); *fhs*, formyl-tetrahydrofolate (HCO-THF) synthase; *folD*, methylene-tetrahydrofolate (CH_2-THF) dehydrogenase/cyclohydrolase; *glyA*, glycine hydroxymethyltransferase; *metF*, methylene-tetrahydrofolate reductase (MTHFR); *metE*/*metH*, methionine synthase.

bidirectional, the previous experiments were insufficient to distinguish whether the enzyme functions are truly missing or actually occur only in the reverse direction or are encoded by genes with unknown sequences (1). In strain 195, expression of both the transcripts and proteins corresponding to the identified genes of the Wood–Ljungdahl pathway has been detected under a variety of growth conditions, suggesting metabolic utility of this pathway (19–21).

All *D. mccartyi* strains identified thus far exhibit common metabolic restrictions; that is, all require organohalide compounds as electron acceptors, hydrogen as electron donor, acetate for biosynthesis, and corrinoids as coenzymes (13). However, even when all known metabolic requirements are met, the growth of *D. mccartyi* has been found to be less robust in axenic cultures than in defined consortia and communities (20–23), for reasons that remain elusive. Because the incomplete Wood–Ljungdahl pathway is present in all sequenced *D. mccartyi* strains and is constantly expressed in strain 195, we hypothesized that it plays an important role in C1 compound and acetyl-CoA metabolism, and that its cellular functionality may be necessary for the growth of *D. mccartyi*. In this study, we applied ^{13}C-tracer experiments to study the Wood–Ljungdahl pathway of strain 195 and used sensitive analytical techniques to detect and quantify CO and CO_2 generated from this pathway. We also evaluated the effects of CO on the growth of strain 195 in isolation and in defined coculture. Finally, we performed a bioinformatics analysis to examine the presence of similarly incomplete Wood–Ljungdahl pathways in other sequenced bacteria and archaea.

Results

Identification of the Functional Elements of the Wood–Ljungdahl Pathway. To investigate the potential functionality of specific reactions within the Wood–Ljungdahl pathway, we examined the ability of strain 195 to use formate as a supplementary carbon source by growing strain 195 with [2-^{13}C]acetate and unlabeled formate with a H_2/CO_2 headspace, and quantified the isotopomers as mass fractions (i.e., M0, M1, M2), representing unlabeled, singly ^{13}C-labeled, and doubly ^{13}C-labeled amino acids, respectively (24). Therefore, if the unlabeled formate was used as a supplemental source of carbon by strain 195, the ^{13}C-labeling profiles of amino acids synthesized from C1 intermediates would be diluted with the unlabeled carbon (25). Analysis of isotopomer distribution of amino acids (Fig. 2 and Table S1) showed that although 94% of serine was singly labeled (M1 = 0.94) in the samples fed [2-^{13}C]acetate alone (16), only 75% of the serine was singly labeled (25% unlabeled, M0 = 0.25) when unlabeled formate was amended to the culture, clearly demonstrating incorporation of the exogenous formate into serine synthesis. In contrast, significant dilution was not observed in the other amino acids and was notably not detected in methionine (M2 = 0.96 and 0.93 in the samples fed [2-^{13}C]acetate alone and [2-^{13}C]acetate and unlabeled formate, respectively), which is biosynthesized using CH_3-THF as a precursor.

To confirm this pattern, the isotopomer distribution was also analyzed for cultures grown with [^{13}C]formate and unlabeled acetate. Similarly, 22% of the serine was singly labeled, whereas 78% was unlabeled, indicating that formate was incorporated into serine, and methionine remained unlabeled

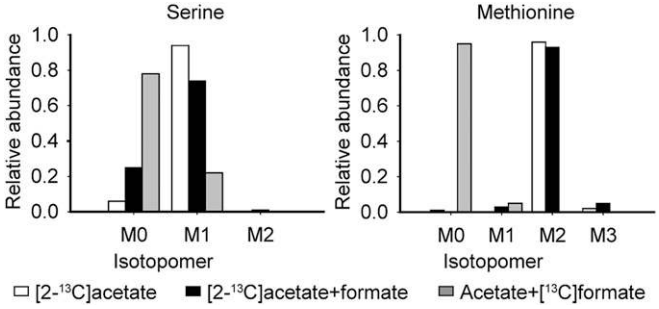

Fig. 2. Relative abundance of different mass isotopomers for serine and methionine in strain 195 cultures grown on [2-^{13}C]acetate with or without unlabeled formate or unlabeled acetate with [^{13}C]formate. The technical variance is less than 2% in duplicated samples for mass isotopomer analysis (16).

(M0 = 0.95) (Fig. 2 and Table S1). Examination of the labeled carbon position in serine revealed that ^{13}C-carbon from formate was only incorporated into the R side chain of the serine molecule (Table S2). This labeling pattern clearly rules out the incorporation of ^{13}C via pyruvate formate lyase (EC 2.3.1.54) (i.e., acetyl-CoA+formate → pyruvate →→→→→→ serine), which should result in serine labeled at the carboxyl group. Instead, the integration of formate carbon in serine is presumably achieved via the portion of the methyl branch of the Wood–Ljungdahl pathway (DET 0671/0707, DET0668/0702) that involves a formate activation by formyl-tetrahydrofolate (HCO-THF) synthase and sequential reduction reactions catalyzed by a bifunctional CH_2-THF cyclohydrolase/dehydrogenase (Fig. 1). This labeling pattern is consistent with CH_2-THF donating the methylene group to a glycine to synthesize a serine via the glycine hydroxymethyltransferase (DET0436) (Fig. 1). However, the further reduction from CH_2-THF to CH_3-THF does not occur because carbon from formate was not incorporated into methionine, indicating that as predicated by the genome annotation, strain 195 lacks a functional MTHFR to reduce CH_2-THF to CH_3-THF (Fig. 1).

When cultures were fed [2-^{13}C]acetate and unlabeled pyruvate, analysis of isotopomer distribution shows that strain 195 incorporated unlabeled carbons from pyruvate for biosynthesis of methionine and aspartate. That is, the doubly labeled methionine decreased (M2 = 0.71) and the singly labeled fraction increased (M1 = 0.23), and for aspartate, the singly labeled decreased (M1 = 0.75) and the unlabeled fraction increased (M0 = 0.23) (Fig. 3*A* and Tables S1 and S3). Our previous study showed that when strain 195 was fed [2-^{13}C]acetate alone, the third carbon in aspartate (M1 = 0.97) and the third and fifth carbons in methionine (M2 = 0.96) were labeled with ^{13}C (16). Because the first four carbons for methionine biosynthesis are derived from aspartate and the fifth comes from CH_3-THF, the similarity in patterns of M1/M2 for methionine and M0/M1 for aspartate suggests that all of the unlabeled carbon from pyruvate that was incorporated in methionine came from aspartate rather than CH_3-THF. That is, if the unlabeled pyruvate contributed any carbon to CH_3-THF, the fifth carbon on the methionine would become unlabeled, creating a mismatched labeling pattern between the aspartate and methionine. Therefore, CH_3-THF was not derived from pyruvate, but rather was derived from the labeled second carbon of [2-^{13}C]acetate through acetyl-CoA (Fig. 3*B*). In addition, *D. mccartyi* is predicted to possess a pyruvate:ferredoxin oxidoreductase [PFOR, EC 1.2.7.1 (DET0724-0727)] that typically functions in either direction to catalyze the oxidative decarboxylation of pyruvate to form acetyl-CoA or reductive carboxylation of acetyl-CoA to form pyruvate (26) (Fig. 3*B*). For the cultures fed [2-^{13}C]acetate and unlabeled pyruvate, unlabeled (M0 = 0.24) alanine (derived from pyruvate) showed similar mass fractions to singly labeled (M1 = 0.23) glutamate (derived from pyruvate and acetyl-CoA), demonstrating that PFOR only functions as a unidirectional enzyme (acetyl-CoA + CO_2 → pyruvate) (Fig. 3*C* and Table S3). This finding is consistent with previous observations that pyruvate cannot serve as sole carbon source for strain 195 (27).

We also tested the ability of strain 195 to take up and incorporate other C1 compounds and tricarboxylic acid cycle intermediates, including methyl-THF, methyl chloride, methyl iodide, methyl thiol, dimethyl sulfide, and trimethyl amine, as well as malate, fumarate, succinate, and citrate. Results indicate that strain 195 is unable to grow on or incorporate any of these compounds under the provided experimental conditions (Tables S4 and S5, no data shown for the cultures incapable of dechlorination and growth).

Cleavage of Acetyl-CoA for Methionine Biosynthesis. The missing MTHFR function in *D. mccartyi* poses an interesting question about the origin of the methyl group in methionine. Exogenous CH_3-THF was not used by strain 195 for methionine biosynthesis when provided with [2-^{13}C]acetate in a H_2/CO_2 headspace, suggesting that CH_3-THF has to be formed endogenously (Table S4).

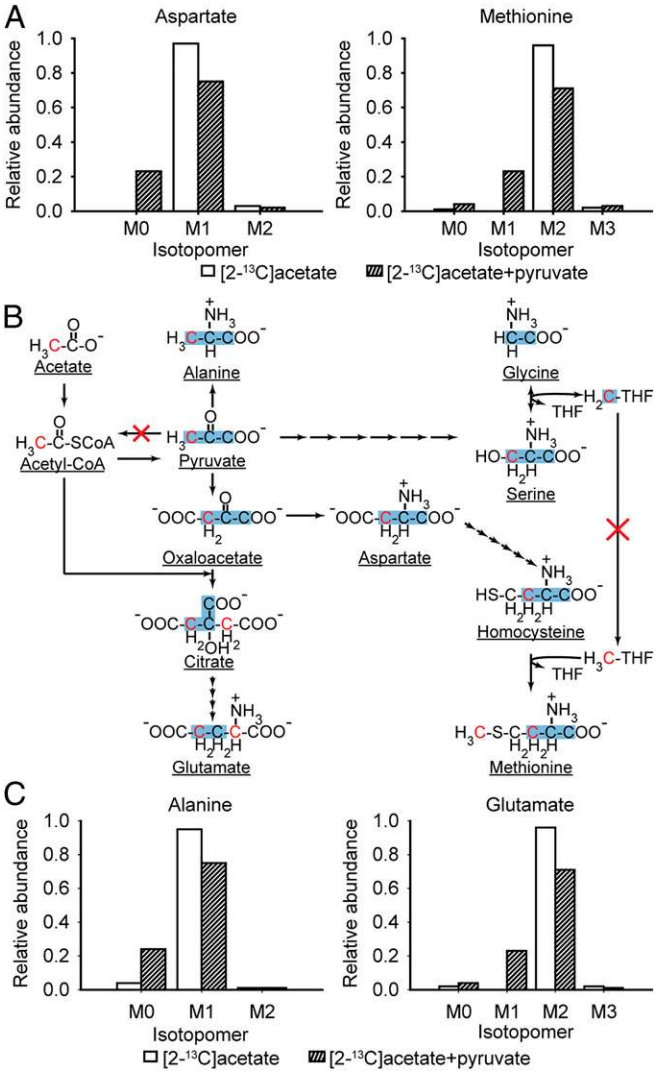

Fig. 3. Isotopomer analyses of amino acid generation from [2-^{13}C]acetate and unlabeled pyruvate in strain 195. (*A*) Relative abundance of different mass isotopomers for aspartate and methionine for strain 195 grown on [2-^{13}C]acetate with or without unlabeled pyruvate. (*B*) Schematic of methionine biosynthesis using aspartate and CH_3-THF as precursors and carbon integration into alanine and glutamate from pyruvate and acetate. ^{13}C-labeled carbon originating from [2-^{13}C]acetate is shown in red. Colored boxes indicate the carbons derived from pyruvate. (*C*) Relative abundance of isotopomers for alanine and glutamate in strain 195 cultures grown on [2-^{13}C]acetate with unlabeled pyruvate.

To confirm that the methyl group of methionine comes from acetyl-CoA cleavage by ACS (Fig. 1) as part of the incomplete Wood–Ljungdahl pathway, we examined whether CO or CO_2 was liberated and released from the carbonyl group of acetyl-CoA using cultures fed with unlabeled, [1-^{13}C]acetate, or [2-^{13}C]acetate.

Cultures fed [1-^{13}C]acetate generated CO that was almost exclusively labeled with ^{13}C (atomic percentage >90%) (Fig. 4*A*). In contrast, less than 5% of the generated CO was labeled in the [2-^{13}C]acetate and unlabeled acetate cultures, indicating that CO is generated from the carbonyl group of acetyl-CoA. Carbon isotope compositions of CO_2 in these experiments exhibited negligible differences for the cultures grown with unlabeled acetate, [1-^{13}C]acetate, or [2-^{13}C]acetate, confirming that CODH and FDH, the two enzymes potentially responsible for oxidizing CO and formate to CO_2, are missing in strain 195, as predicted in the genome annotation (12) (Fig. 1).

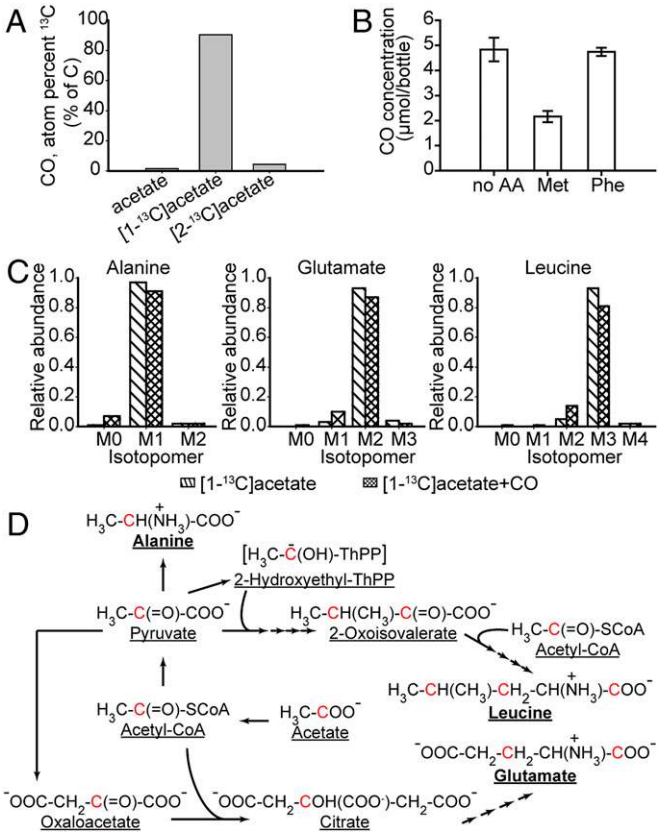

Fig. 4. CO released and incorporated by strain 195. (*A*) ^{13}CO was dominant in the cultures amended with [1-^{13}C]acetate, but not in cultures amended with either unlabeled or [2-^{13}C]acetate. (*B*) CO quantified in strain 195 cultures grown in the presence of methionine (Met), no amino acid (no AA), or phenylalanine (Phe). (*C*) Relative abundance of isotopomers for alanine, glutamate and leucine in strain 195 cultures grown on [1-^{13}C]acetate with or without unlabeled CO. (*D*) Biosynthesis of alanine, glutamate and leucine from acetyl-CoA. ThPP, thiamine diphosphate.

Our previous study demonstrated that strain 195 can uptake and incorporate more than 30% of its proteinogenic methionine from the extracellular environment (25). To further confirm that CO is a by-product of the acetyl-CoA cleavage reaction for methionine biosynthesis, we compared the CO concentrations of cultures amended with methionine to those without methionine. As a positive control, cultures provided with phenylalanine were also analyzed for their CO concentrations, because exogenous phenylalanine can also be imported and integrated into strain 195 biomass but does not play a role in methionine synthesis (25). We reasoned that if strain 195 cleaves acetyl-CoA to generate the methyl group for methionine biosynthesis, then providing exogenous methionine for import would decrease the need for its synthesis, resulting in decreased CO production. As expected, the cultures amended with methionine produced ~50% less CO than the cultures without amendment or with phenylalanine after five doses of TCE amendment (Fig. 4*B*).

Because ACS is often a bidirectional enzyme, we also tested whether CO can be incorporated via acetyl-CoA synthesis in the partial Wood–Ljungdahl pathway (28). We compared isotopomer distribution of three amino acids (alanine, glutamate, and leucine) between cultures fed [1-^{13}C]acetate alone and those fed [1-^{13}C]acetate with unlabeled CO. Approximately 6~10% dilution by the unlabeled carbon from CO was detected in the three amino acids that contain one to three carbons derived from the carbonyl group of acetyl-CoA (Fig. 4 *C* and *D* and Table S6), indicating that CO can be incorporated into the central metabolism even when present in minor amounts, presumably via ACS.

CO Accumulation in Strain 195 Isolates and Cocultures. We compared CO accumulation in strain 195 isolates with accumulation in cocultures of strain 195 and *Desulfovibrio vulgaris* Hildenborough (DvH) over consecutive feeding doses of TCE (Fig. 5*A*). We found that CO was generated and accumulated during the growth of the strain 195 isolate, eventually reaching 5 μmol per bottle after five doses of TCE (totaling 385 μmol) were completely transformed. TCE dechlorination activity ceased during the sixth dose of amended TCE as previously observed (20). In contrast, although small amounts of CO accumulated in the strain 195/DvH coculture, it remained around 0.5 μmol per bottle, and did not increase even after nine consecutive doses (totaling 693 μmol), and the coculture continued to support TCE dechlorination over 15 doses (Fig. 5*A* and Fig. S1).

To understand the potential inhibitory effects of CO on strain 195, we examined the growth of strain 195 in a titration experiment with exposure to different amounts of CO after one dose of TCE (Fig. 5*B*). We found that CO concentrations amended as low as 0.8 μmol per bottle exerted slight adverse effects on strain 195's growth, whereas amended concentrations of 6–10 μmol per bottle exerted moderate to severe inhibition, with cell numbers significantly decreasing over three consecutive TCE doses.

Bioinformatic Analyses of MTHFR and ACS Genes in Sequenced Microbial Genomes. Because the substitution of missing MTHFR function by acetyl-CoA cleavage has not been previously reported, we conducted a bioinformatics analyses on the sequenced bacterial and archaeal genomes to determine whether this characteristic is present in other microorganisms. We first identified the genomes without an annotated MTHFR gene. Of 2,277 bacterial and archaeal genomes in the National Center for Biotechnology Information (NCBI) genomes database (as of February of 2013), 1,548 were found to have annotated MTHFR genes (Dataset S1). A blastx search comparing the remaining 729 genomes to the annotated MTHFR protein sequences identified an additional 303 genomes containing MTHFR homologous genes, and another seven genomes with MTHFR genes were identified by manual curation (Dataset S1). MTHFR genes were not identified in 419 genomes (Dataset S1). Some of these genomes belonged to parasitic or symbiotic organisms, whose close association with a host may explain their absence.

Further analysis of the 419 genomes without MTHFR genes focused on the presence of an ACS gene. Within this group, homologs of this gene were only found in sequenced *D. mccartyi* strains, but not in other genomes. In addition, all *D. mccartyi* strains were found to be missing gene homologs of bacterial betaine-homocysteine methyltransferase, proteins that catalyze a methyl transfer reaction from betaine instead of CH_3-THF for methionine biosynthesis.

Discussion

Compared with identified functionalities of the Wood–Ljungdahl pathway in homoacetogens, sulfate-reducers, and methanogens, little is known regarding the metabolic role of the incomplete

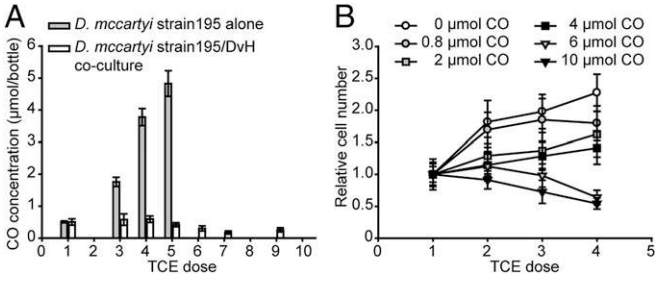

Fig. 5. (*A*) CO generation during dechlorination of multiple TCE doses by strain 195 in pure cultures and in cocultures with DvH. (*B*) Inhibitory effects of different CO concentrations on strain 195 growth.

Wood–Ljungdahl pathway in organohalide-respiring *D. mccartyi* (1, 3). It has also been unknown how *D. mccartyi* strains without identified *metF* homologs achieve methylation of homocysteine for methionine biosynthesis. In this study, we demonstrated that although the Wood–Ljungdahl pathway in *D. mccartyi* can assimilate formate and to a lesser extent CO in certain amino acids, the most important role of this pathway is to cleave acetyl-CoA for CH_3-THF generation to substitute for the missing MTHFR function in methionine biosynthesis. Others have previously suggested that some soil and marine bacteria use an alternative methionine biosynthesis pathway using betaine instead of CH_3-THF as the methyl donor to homocysteine via the activities of betaine-homocysteine methyltransferase (29–32). However, our analysis does not support this possibility in *D. mccartyi* because bioinformatics analysis indicates lack of a homolog to betaine-homocysteine methyltransferase in all five sequenced *D. mccartyi* strains. Moreover, ^{13}C-experiments distinctly demonstrated that acetyl-CoA cleavage is the origin of the methyl group of methionine, evidenced by the comparison of ^{13}C-labeling profile of methionine and aspartate (Fig. 3*B*), as well as reduced CO production in the presence of exogenous methionine (Fig. 4*B*). Although variations in C1 metabolism, such as the replacement of tetrahydrofolate by polyglutamate or methanopterins and NAD(P)H instead of ferredoxin as the cofactor for MTHFR (2, 33, 34), have previously been reported for bacteria and archaea, to our knowledge, the complete replacement of the MTHFR function with acetyl-CoA cleavage is novel. Although our bioinformatics analysis suggested that this strategy for generating CH_3-THF is not found in other sequenced bacteria and archaea, it is still unclear whether this strategy has wider distribution in the environment, given the limited numbers of sequenced organisms and the inherent challenges associated with growing CO-generating organisms in isolation.

In addition to its pivotal roles in methionine biosynthesis, the incomplete Wood–Ljungdahl pathway of *D. mccartyi* was also found to affect the growth of *D. mccartyi* in axenic cultures through the generation of CO, an obligate by-product of acetyl-CoA cleavage. Our analysis revealed that CO accumulated in the headspace of strain 195 axenic cultures because of the lack of CODH function and caused slight to severe inhibition to growth (Fig. 5). The inhibition may be because of adverse effects of CO on metalloenzymes, such as hydrogenases, with which CO can competitively form stable complexes that block the interactions of substrate and enzymes (35). Compared with the reported CO concentrations [1–2%, 7.5%, and 50–100% (vol/vol)] that cause severe inhibition to other anaerobes with hydrogenases, the severe CO toxicity level of strain 195 [6 μmol per bottle, (~0.1%) (vol/vol)] is orders-of-magnitudes lower (36, 37). This extreme CO toxicity is likely one reason that growth of axenic *D. mccartyi* cultures is observably unreliable (20–23).

Fortunately, the adverse effects of CO toxicity on *D. mccartyi* growth and dechlorination can be ameliorated by the presence of CO-oxidizing organisms, as demonstrated in our experiments with the coculture of strain 195 and DvH. In fact, we propose that CO might be an important substance exchanged between *D. mccartyi* and its coinhabitants within microbial communities. Because of the low redox potential (E_0') of the CO_2/CO couple (−524 mV), CO oxidation can serve as an excellent source of energy for anaerobic microorganisms (38, 39). CO oxidation has been observed in anaerobic organisms with various respiratory processes, including sulfate-reducers, hydrogen-producers, homoacetogens, and methanogens that have commonly been found to coexist with *D. mccartyi* in diverse environments (40–42). Consequently, bacteria and archaea capable of CO-oxidization could gain additional energy from coexistence with *D. mccartyi*, while enhancing the robust growth of *D. mccartyi*, representing another potentially important substrate for interspecies transfer and syntrophic interactions between *D. mccartyi* and other community members.

Analysis of the acetyl-CoA cleavage activity of *D. mccartyi* indicates that the reaction is catalyzed by a monomeric ACS, rather than a bifunctional heteromeric enzyme complex, CODH/ACS, indicating that the ACS function can be separate from CODH. Indeed, the activity of a monomeric ACS has been previously demonstrated in *Carboxydothermus hydrogenoformans* grown under excess CO concentrations, where ACS showed a comparable specific activity to CODH/ACS in acetyl-CoA cleavage or synthesis (8). Like the monomeric ACS in *C. hydrogenoformans*, the ACS of *D. mccartyi* is also likely a bidirectional enzyme, but with a more prevalent in vivo acetyl-CoA cleavage activity than acetyl-CoA synthesis from CO, a methyl group and CoA (Figs. 1 and 4 *C* and *D*). In addition, although *D. mccartyi* lacks a gene homolog of MeTr (*acsE*), the methyl transfer reaction from CFeSP to CH_3-THF is actually active. It is possible that the methyl transferase is encoded by a gene dissimilar to known *acsE* genes or is catalyzed by a multienzyme complex containing ACS and CFeSP if the *D. mccartyi* ACS is an analog to archaeal acetyl-CoA decarbonylase/synthase (43, 44). The uniqueness of the ACS in *D. mccartyi* calls for the further characterization of this interesting enzyme, which may lead us to a better understanding of the evolution of the Wood–Ljungdahl pathway.

In summary, we demonstrated the crucial metabolic roles of the incomplete Wood–Ljungdahl pathway in central metabolism of the obligately organohalide-respiring *D. mccartyi*. The knowledge of metabolic functionalities gained in this study improves our understanding of the central metabolism of environmentally important bacteria, and will better equip us to study the ecological distribution and impact of incomplete Wood–Ljungdahl pathways in other environmental microorganisms.

Materials and Methods

Bacterial Strain and Culture Conditions. Strain 195 was grown aseptically in batch cultures at 34 °C with a defined mineral salt medium and a H_2/CO_2 headspace (80/20 vol/vol), as described previously (16). The medium was amended with 2 mM sodium acetate, liquid TCE (~77 μmol per bottle), and a modified Wolin vitamin solution containing 37 nM B_{12}. To analyze the incorporation of exogenous unlabeled carbons, 2 mM of each organic acid (i.e., formate, pyruvate, citrate, succinate, fumarate, and malate) or 2 mM of each C1 compound (i.e., dimethyl sulfate, trimethyl amine, methyl chloride, methyl iodide, and methyl thiol) or 0.5 mM CH_3-THF or 5 μM CO was amended together with 2 mM ^{13}C-labeled sodium acetate into the culture medium. To minimize unlabeled carbon introduced from inoculation, strain 195 biomass was subcultured with 2% (vol/vol) inoculum in labeled medium three times before being harvested for isotopomer analysis. A coculture of strain 195 and DvH was grown in the same medium with the substitutions of 5 mM lactate and N_2/CO_2 (90/10 vol/vol) headspace for acetate and H_2/CO_2 headspace (21).

Analytical Methods. Approximately 1.5 L of liquid culture (~7.7×10^7 cells/mL) was aseptically harvested by centrifugation at 22,000 × *g* for 15 min at 4 °C. The cell pellet was washed three times and stored at –80 °C before use. The preparation and isotopomeric analysis of proteogenic amino acids were performed as previously described (45). Details of biomass hydrolysis, derivatization of amino acids and GC-MS analysis are present in *SI Materials and Methods*. Isotopomer data correction and analysis were conducted as described by Wahl et al. (46). The isotopic labeling data were shown as mass fractions (i.e., M0, M1, M2. . .) representing amino acids containing unlabeled, singly ^{13}C-labeled, and doubly ^{13}C-labeled isotopomers, respectively (24).

Ethenes in culture headspace were measured using a GC (Hewlett-Packard model 5890; Agilent Technologies) with a GC-GasPro capillary column (30 m × 0.32 mm, particle-free PLOT phase; J&W Scientific) and a flame ionization detector, as described previously (16). CO concentrations and CO isotopic compositions were measured using a gas chromatograph isotope ratio mass spectrometer (Thermo Fisher Scientific). Briefly, 300–1,000 μL of headspace sample was taken from sample vials and injected to 25- to 250-μL volume stainless steel loops mounted on a six-port valve (Valco Instruments). Samples were transferred into the gas chromatograph by switching the six-port valve. CO was separated chromatographically on a HP-Molsieve fused silica capillary column (30 m × 0.32 mm, 12-μm film thickness; Agilent Technologies). The stable isotope abundance is reported in atom percent ^{13}C. CO concentrations were determined using peaks area of mass 28, 29, and 30.

Cell numbers of the cultures were determined by quantitative real-time PCR with primers specific to the strain 195 *tceA* or 16S rRNA gene, using a StepOnePlus real-time PCR system (Applied Biosystems) as previously described (21, 25). Relative cell numbers are reported as multiples of the average levels observed in cultures after one dose of TCE.

Bioinformatics Analyses of Genes Coding MTHFR, ACS, and Betaine-Homocysteine Methyltransferase. A bioinformatic analysis was performed to evaluate the prevalence of MTHFR genes in sequenced microbial genomes and to identify organisms lacking this gene. The search was performed using all bacterial and archaeal genomes in the NCBI genomes database, downloaded in February of 2013. Detailed bioinformatics analyses of MTHFR genes are present in *SI Materials and Methods*. In the genomes that lack of MTHFR genes, the presence of ACS (EC 2.3.1.169) genes was searched to assess the distribution of the incomplete Wood–Ljungdahl pathway in other prokaryotes (47). Finally, all *D. mccartyi* strains were searched for the homologs of betaine-homocysteine methyltransferase (EC 2.1.1.5) using the bacterial protein sequences found in BRENDA (in August of 2013) and reported elsewhere (31).

ACKNOWLEDGMENTS. We thank John W. Cunha and Jeffrey T. Kwan for laboratory assistance. This work was supported by the National Institute on Environmental Health Sciences Superfund Basic Research Program P42ES04705-19 (to L.A.-C.); National Science Foundation CBET1336709 (to L.A.-C.); the Strategic Environmental Research and Development Program ER-1587 (to L.A.-C.); and National Science Foundation Career Grant MCB0954016 (to Y.J.T.).

1. Ragsdale SW, Pierce E (2008) Acetogenesis and the Wood-Ljungdahl pathway of CO_2 fixation. *Biochim Biophys Acta* 1784(12):1873–1898.
2. Fuchs G (2011) Alternative pathways of carbon dioxide fixation: Insights into the early evolution of life? *Annu Rev Microbiol* 65:631–658.
3. Drake HL, Gössner AS, Daniel SL (2008) Old acetogens, new light. *Ann N Y Acad Sci* 1125:100–128.
4. Fuchs G (1995) Variations of the acetyl-CoA pathway in diversely related microorganisms that are not acetogens. *Acetogenesis*, Chapman & Hall Microbiology Series, ed Drake H (Springer, New York), pp 507–520.
5. Ladapo J, Whitman WB (1990) Method for isolation of auxotrophs in the methanogenic archaebacteria: Role of the acetyl-CoA pathway of autotrophic CO_2 fixation in *Methanococcus maripaludis*. *Proc Natl Acad Sci USA* 87(15):5598–5602.
6. Ljungdahl LG (1986) The autotrophic pathway of acetate synthesis in acetogenic bacteria. *Annu Rev Microbiol* 40:415–450.
7. Schouten S, et al. (2004) Stable carbon isotopic fractionations associated with inorganic carbon fixation by anaerobic ammonium-oxidizing bacteria. *Appl Environ Microbiol* 70(6):3785–3788.
8. Svetlitchnyi V, et al. (2004) A functional Ni-Ni-[4Fe-4S] cluster in the monomeric acetyl-CoA synthase from *Carboxydothermus hydrogenoformans*. *Proc Natl Acad Sci USA* 101(2):446–451.
9. Wood HG (1991) Life with CO or CO_2 and H_2 as a source of carbon and energy. *FASEB J* 5(2):156–163.
10. Kube M, et al. (2005) Genome sequence of the chlorinated compound-respiring bacterium *Dehalococcoides* species strain CBDB1. *Nat Biotechnol* 23(10):1269–1273.
11. McMurdie PJ, et al. (2009) Localized plasticity in the streamlined genomes of vinyl chloride respiring *Dehalococcoides*. *PLoS Genet* 5(11):e1000714.
12. Seshadri R, et al. (2005) Genome sequence of the PCE-dechlorinating bacterium *Dehalococcoides ethenogenes*. *Science* 307(5706):105–108.
13. Löffler FE, et al. (2013) *Dehalococcoides mccartyi* gen. nov., sp. nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation, belong to a novel bacterial class, *Dehalococcoidia* classis nov., order *Dehalococcoidales* ord. nov. and family *Dehalococcoidaceae* fam. nov., within the phylum *Chloroflexi*. *Int J Syst Evol Microbiol* 63(Pt 2):625–635.
14. Rüdiger H, Jaenicke L (1973) The biosynthesis of methionine. *Mol Cell Biochem* 1(2): 157–168.
15. He JZ, Holmes VF, Lee PKH, Alvarez-Cohen L (2007) Influence of vitamin B_{12} and cocultures on the growth of *Dehalococcoides* isolates in defined medium. *Appl Environ Microbiol* 73(9):2847–2853.
16. Tang YJ, et al. (2009) Investigation of carbon metabolism in "*Dehalococcoides ethenogenes*" strain 195 by use of isotopomer and transcriptomic analyses. *J Bacteriol* 191(16):5224–5231.
17. Rückert C, Pühler A, Kalinowski J (2003) Genome-wide analysis of the L-methionine biosynthetic pathway in *Corynebacterium glutamicum* by targeted gene deletion and homologous complementation. *J Biotechnol* 104(1-3):213–228.
18. Marco-Urrea E, Seifert J, von Bergen M, Adrian L (2012) Stable isotope peptide mass spectrometry to decipher amino acid metabolism in *Dehalococcoides* strain CBDB1. *J Bacteriol* 194(16):4169–4177.
19. Johnson DR, Nemir A, Andersen GL, Zinder SH, Alvarez-Cohen L (2009) Transcriptomic microarray analysis of corrinoid responsive genes in *Dehalococcoides ethenogenes* strain 195. *FEMS Microbiol Lett* 294(2):198–206.
20. Johnson DR, et al. (2008) Temporal transcriptomic microarray analysis of "*Dehalococcoides ethenogenes*" strain 195 during the transition into stationary phase. *Appl Environ Microbiol* 74(9):2864–2872.
21. Men Y, et al. (2012) Sustainable syntrophic growth of *Dehalococcoides ethenogenes* strain 195 with *Desulfovibrio vulgaris* Hildenborough and *Methanobacterium congolense*: Global transcriptomic and proteomic analyses. *ISME J* 6(2):410–421.
22. Ahsanul Islam M, Edwards EA, Mahadevan R (2010) Characterizing the metabolism of *Dehalococcoides* with a constraint-based model. *PLOS Comput Biol* 6(8):e1000887.
23. Yan J, Ritalahti KM, Wagner DD, Löffler FE (2012) Unexpected specificity of interspecies cobamide transfer from *Geobacter spp.* to organohalide-respiring *Dehalococcoides mccartyi* strains. *Appl Environ Microbiol* 78(18):6630–6636.
24. You L, et al. (2012) Metabolic pathway confirmation and discovery through ^{13}C-labeling of proteinogenic amino acids. *J Vis Exp* (59):e3583.
25. Zhuang W-Q, et al. (2011) Selective utilization of exogenous amino acids by *Dehalococcoides ethenogenes* strain 195 and its effects on growth and dechlorination activity. *Appl Environ Microbiol* 77(21):7797–7803.
26. Ragsdale SW (2003) Pyruvate ferredoxin oxidoreductase and its radical intermediate. *Chem Rev* 103(6):2333–2346.
27. Maymó-Gatell X, Chien Y-T, Gossett JM, Zinder SH (1997) Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science* 276(5318):1568–1571.
28. Hennig SE, Jeoung JH, Goetzl S, Dobbek H (2012) Redox-dependent complex formation by an ATP-dependent activator of the corrinoid/iron-sulfur protein. *Proc Natl Acad Sci USA* 109(14):5235–5240.
29. Barra L, et al. (2006) Interrelations between glycine betaine catabolism and methionine biosynthesis in *Sinorhizobium meliloti* strain 102F34. *J Bacteriol* 188(20): 7195–7204.
30. Sowell SM, et al. (2008) Proteomic analysis of stationary phase in the marine bacterium "*Candidatus Pelagibacter ubique*". *Appl Environ Microbiol* 74(13):4091–4100.
31. Rodionov DA, Vitreschak AG, Mironov AA, Gelfand MS (2004) Comparative genomics of the methionine metabolism in Gram-positive bacteria: A variety of regulatory systems. *Nucleic Acids Res* 32(11):3340–3353.
32. Hug LA, Beiko RG, Rowe AR, Richardson RE, Edwards EA (2012) Comparative metagenomics of three *Dehalococcoides*-containing enrichment cultures: The role of the non-dechlorinating community. *BMC Genomics* 13:327.
33. Schauder R, Preuß A, Jetten M, Fuchs G (1988) Oxidative and reductive acetyl CoA/carbon monoxide dehydrogenase pathway in *Desulfobacterium autotrophicum*. *Arch Microbiol* 151(1):84–89.
34. Thauer RK, Kaster AK, Seedorf H, Buckel W, Hedderich R (2008) Methanogenic archaea: Ecologically relevant differences in energy conservation. *Nat Rev Microbiol* 6(8):579–591.
35. Ragsdale SW (2004) Life with carbon monoxide. *Crit Rev Biochem Mol Biol* 39(3): 165–195.
36. Parshina SN, Sipma J, Henstra AM, Stams AJ (2010) Carbon monoxide as an electron donor for the biological reduction of sulphate. *Int J Microbiol* 2010:319527.
37. Meuer J, Bartoschek S, Koch J, Künkel A, Hedderich R (1999) Purification and catalytic properties of Ech hydrogenase from *Methanosarcina barkeri*. *Eur J Biochem* 265(1): 325–335.
38. Grahame DA, DeMoll E (1995) Substrate and accessory protein requirements and thermodynamics of acetyl-CoA synthesis and cleavage in *Methanosarcina barkeri*. *Biochemistry* 34(14):4617–4624.
39. Thauer RK (1988) Citric-acid cycle, 50 years on. Modifications and an alternative pathway in anaerobic bacteria. *Eur J Biochem* 176(3):497–508.
40. Duhamel M, Edwards EA (2006) Microbial composition of chlorinated ethene-degrading cultures dominated by *Dehalococcoides*. *FEMS Microbiol Ecol* 58(3):538–549.
41. Men Y, Lee PK, Harding KC, Alvarez-Cohen L (2013) Characterization of four TCE-dechlorinating microbial enrichments grown with different cobalamin stress and methanogenic conditions. *Appl Microbiol Biotechnol* 97(14):6439–6450.
42. Oelgeschläger E, Rother M (2008) Carbon monoxide-dependent energy metabolism in anaerobic bacteria and archaea. *Arch Microbiol* 190(3):257–269.
43. Abbanat DR, Ferry JG (1991) Resolution of component proteins in an enzyme complex from *Methanosarcina thermophila* catalyzing the synthesis or cleavage of acetyl-CoA. *Proc Natl Acad Sci USA* 88(8):3272–3276.
44. Ferry JG (1999) Enzymology of one-carbon metabolism in methanogenic pathways. *FEMS Microbiol Rev* 23(1):13–38.
45. Tang YJ, Hwang JS, Wemmer DE, Keasling JD (2007) *Shewanella oneidensis* MR-1 fluxome under various oxygen conditions. *Appl Environ Microbiol* 73(3):718–729.
46. Wahl SA, Dauner M, Wiechert W (2004) New tools for mass isotopomer data evaluation in ^{13}C flux analysis: Mass isotope correction, data consistency checking, and precursor relationships. *Biotechnol Bioeng* 85(3):259–268.
47. Pierce E, et al. (2008) The complete genome sequence of *Moorella thermoacetica* (f. *Clostridium thermoaceticum*). *Environ Microbiol* 10(10):2550–2573.