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Complete Genome Sequence of the Dehalorespiring Bacterium *Desulfitobacterium hafniense* Y51 and Comparison with *Dehalococcoides ethenogenes* 195

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Desulfitobacterium **strains have the ability to dechlorinate halogenated compounds under anaerobic conditions by dehalorespiration. The complete genome of the tetrachloroethene (PCE)-dechlorinating strain *Desulfitobacterium hafniense* Y51 is a 5,727,534-bp circular chromosome harboring 5,060 predicted protein coding sequences. This genome contains only two reductive dehalogenase genes, a lower number than reported in most other dehalorespiring strains. More than 50 members of the dimethyl sulfoxide reductase superfamily and 30 paralogs of the flavoprotein subunit of the fumarate reductase are encoded as well. A remarkable feature of the genome is the large number of *O*-demethylase paralogs, which allow utilization of lignin-derived phenyl methyl ethers as electron donors. The large genome reveals a more versatile microorganism that can utilize a larger set of specialized electron donors and acceptors than previously thought. This is in sharp contrast to the PCE-dechlorinating strain *Dehalococcoides ethenogenes* 195, which has a relatively small genome with a narrow metabolic repertoire. A genomic comparison of these two very different strains allowed us to narrow down the potential candidates implicated in the dechlorination process. Our results provide further impetus to the use of desulfitobacteria as tools for bioremediation.**

Halogenated organic compounds are released into the environment from natural and anthropogenic sources. Many anthropogenic halogenated chemicals, like chlorinated haloalkenes (7, 10, 46), benzenes (1), and dioxins (5), are of particular concern due to their toxicity to humans and other forms of life. This toxicity is often paired with high recalcitrance to degradation, especially in anaerobic environments, leading to persistent contamination.

Anaerobic environments are frequently characterized by limited availability of electron acceptors. Theoretical calculations have shown that coupling the reduction of many halogenated organic compounds to the oxidation of suitable substrates is a way to harness energy (46). As determined two decades ago, this source of energy is utilized by the microbial community. The oxidation of available electron donors coupled to the reduction of halogenated organic compounds while energy is conserved is called dehalorespiration (7, 10, 46). Dehalorespiring strains have been isolated independently from contaminated sites around the world. The two most prominent genera resulting from these isolation efforts are *Dehalococcoide*s (29) and *Desulfitobacterium* (51), and various strains of these genera are used as model systems to study dehalorespiration (8, 11, 51).

Dehalococcoides ethenogenes 195 is one of the few strains isolated to date which can dechlorinate tetrachloroethene (PCE) to ethene (29). *D. ethenogenes* 195 can use only hydrogen as an electron donor and chlorinated compounds as electron acceptors (29).

Desulfitobacterium strains are also known to dechlorinate a wide variety of substrates, including halophenolic compounds and chloroalkenes (7, 10, 46). Although several strains can use PCE or trichloroethene (TCE) as an electron acceptor, no *Desulfitobacterium* strain isolated so far completely dechlorinates these compounds to ethene (7, 14, 48). In contrast to *Dehalococcoides* strains, *Desulfitobacterium* strains can utilize electron acceptors other than chlorinated compounds. Several strains that are capable of deiodination (21) and reduction of As(V), Fe(III), Se(VI), Mn(IV), and a variety of oxidized sulfur species (37) have been isolated, although currently little is known about how widespread these capabilities are in this genus.

Since *Desulfitobacterium* and *Dehalococcoides* strains are frequently encountered at contaminated sites, these genera have attracted considerable attention for use as bioremediation agents. The use of these strains in real life, however, is hampered by the lack of information about how the dehalogenation process is embedded in the general metabolism of the organisms and the conditions that allow these microorganisms to proliferate in the environment.

Here we report the first complete genomic sequence of the genus *Desulfitobacterium*. *Desulfitobacterium hafniense* Y51 (formerly *Desulfitobacterium* sp. strain Y51) was isolated from a contaminated site in Japan based on its ability to efficiently dechlorinate PCE even at its highest water solubility (48). The recent publication of the *D. ethenogenes* 195 genomic sequence

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TABLE 1. Comparison of the genomes of two dehalorespiring strains

Parameter	*Desulfitobacterium hafniense* Y51	*Dehalococcoides ethenogenes* 195[a]
Taxonomic position	Clostridia	*Chloroflexi*
Size (bp)	5,727,534	1,469,720
G+C content (%)	47.4	48.9
No. of rRNA operons (16S-23S-5S)	6	1[b]
No. of tRNAs	59	46
No. of predicted CDSs	5,060	1,580
No. of dehalogenase genes	2	19

[a] The accession number is NC002936 (Refseq) or CP000027 (GenBank).
[b] 16S rRNA and 5S-23S rRNAs are located separately on the genome.

(43) allowed us to compare the two sequences and highlight the similarities and differences between the organisms.

MATERIALS AND METHODS

Genome sequencing. *D. hafniense* Y51 was cultured as described previously (48). The genome was sequenced using the whole-genome shotgun method (12). Genomic DNA was isolated using a standard phenol-chloroform extraction-based protocol and was mechanically sheared. Two genomic DNA libraries with average insert sizes of 2 kb and 8 kb were constructed in the pUC118 vector (53). Sequencing was performed using an ABI Prism ABI3730 DNA analyzer (Applied Biosystems). The sequences were base called and assembled using Phred/Phrap/Consed (11, 15). Gaps were closed by primer walking for gap-spanning plasmid clones, direct sequencing of PCR products, and nested PCR-assisted contig extension. Misassemblies and frameshifts were corrected by verifying the positions of repeated DNA regions (rRNA gene, repetitive sequences) or ambiguous DNA regions using PCR. The final genome sequence is based on 98,319 reads. The error rate is 0.04 base per 10 kb as calculated using Consed.

Gene prediction and annotation. rRNA-encoding genomic regions were located by a BLASTN homology search against the 16S rRNA sequence of *D. hafniense* Y51 and the 23S and 5S rRNA sequences of *Thermoanaerobacter tengcongensis* (2). tRNA-encoding regions were predicted by tRNA scan SE (25).

Protein coding sequences (CDS) were predicted by glimmer (39) trained on the whole genome sequence using an open reading frame cutoff value of 240 bp. In order to identify false-positive hits, we compared all glimmer predictions with entries in the Swiss-Prot database and with all coding sequences of completely sequenced organisms (as of 9 July 2005) using BLASTP (e-value, <1e-10). Conflicting coding sequences were removed from the coding sequence list. The remainder of the genome was screened for the presence of CDSs by a BLASTX homology search against CDSs of *Clostridium acetobutylicum* ATCC 824, *Bacillus subtilis* subsp. *subtilis* 168, and *Escherichia coli* K-12. This second step allowed us to identify CDSs missed by glimmer, either because they were shorter than 240 bp or because the signature was not recognized as a coding sequence. The homologous regions identified were extended to CDSs. The start codon of each CDS was manually revised when it was necessary.

Functional annotation of the proteome was carried out by a BLASTP homology search against the NCBI Clusters of Orthologous Groups (COG) database (ftp://ftp.ncbi.nih.gov/pub/COG/old/) (50). Subcellular localization of the coding sequences was predicted by using PSORTb (13).

The homolog of each *D. hafniense* Y51 coding sequence that was most similar to any coding sequence of a completely sequenced genome (as of July 2005) was determined by a BLASTP search using a cutoff value of 1e-4. A small self-written Perl script was used to extract the metadata containing the strain information associated with the highest-similarity hits.

Comparative genomic analysis. The predicted coding sequences of *D. hafniense* Y51 and *D. ethenogenes* 195 were compared to each other by BLASTP using a cutoff value of 1e-4. Reciprocal highest levels of similarity were used to identify a set of 751 orthologous coding sequences. The 751 *D. hafniense* Y51 coding sequences were compared to a sample containing all coding sequences of completely sequenced organisms (as of July 2005), including that of *D. ethenogenes* 195. Conversely, the 751 *D. ethenogenes* 195 coding sequences were compared to a sample containing all coding sequences of completely sequenced organisms (as of July 2005), including that of *D. hafniense* Y51 but not that of *D. ethenogenes* 195. A small self-written Perl script was used to extract the *D. hafniense* Y51 and *D. ethenogenes* 195 coding sequences (and the metadata associated with them) that exhibited the highest levels of similarity to *D. ethenogenes* 195 and *D. hafniense* Y51 coding sequences, respectively.

Nucleotide sequence accession number. The complete *D. hafniense* Y51 genome sequence has been deposited in the DDBJ database under accession no. AP008230.

RESULTS AND DISCUSSION

General features of *D. hafniense* Y51. The *D. hafniense* Y51 genome is a single circular 5,727,534-bp chromosome with 5,060 predicted CDSs (Table 1 and Fig. 1). This strain harbors no plasmids. The replication origin of the chromosome was defined using the position of the transition point of GC skew (Fig. 2) (24, 30) and the presence of the characteristic replication protein encoded by *dnaA*. The GC skew analysis also clearly identified the chromosomal arms. In most prokaryotic organisms the sizes of the two chromosomal arms are usually similar, but in *D. hafninense* Y51 one arm is approximately twice as long as the other. To our knowledge, this is the most extreme case in any completely sequenced microorganism with a circular chromo-

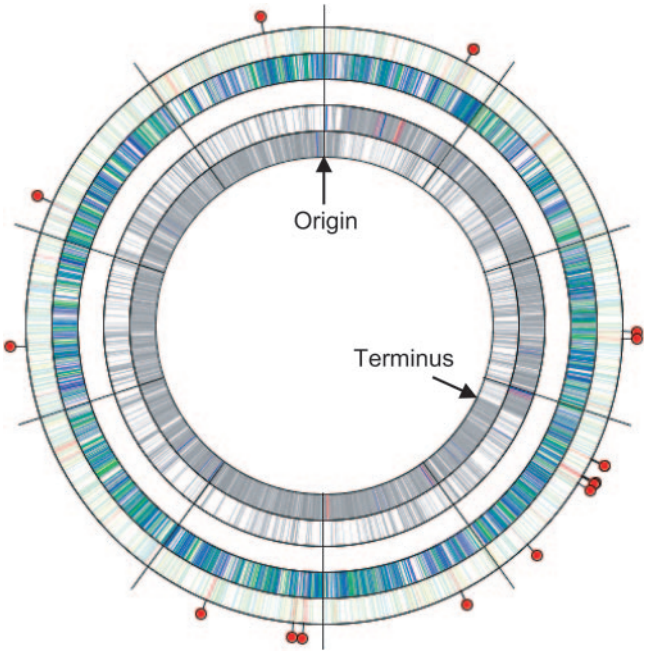

FIG. 1. Schematic circular representation of the *D. hafniense* Y51 genome. The *D. hafniense* Y51 genome is a 5.7-Mbp single circular chromosome. The innermost two circles show the positions of coding sequences as gray bars on the plus and minus strands. Loci encoding rRNA and tRNA are red and blue, respectively. The outermost two circles show the positions of CDSs with similarity (e-value, <1e-4) to CDSs of other prokaryotic microorganisms. The bars are color coded to show the taxonomic affiliation of the organism with the highest similarity hit. For the inner circle the colors are as follows: *Firmicutes* clostridia, green; *Firmicutes* bacilli, blue; other *Firmicutes*, gray. And for the outer circle the colors are as follows: *Proteobacteria*, yellow; *Chloroflexi*, red; bacteria other than *Firmicutes*, *Proteobacteria*, or *Chloroflexi*, light green; and *Archaea*, light blue. Note that the vast majority of *D. hafniense* Y51 CDSs are most similar to CDSs of strains belonging to the clostridia and bacilli, the two groups that are predicted to be the closest relatives of *D. hafniense* Y51 based on taxonomy and 16S rRNA-based phylogeny (data not shown). Red pins indicate the positions of *pceA* and 18 CDSs identified by the comparative genome analysis whose results are shown in Table 6.

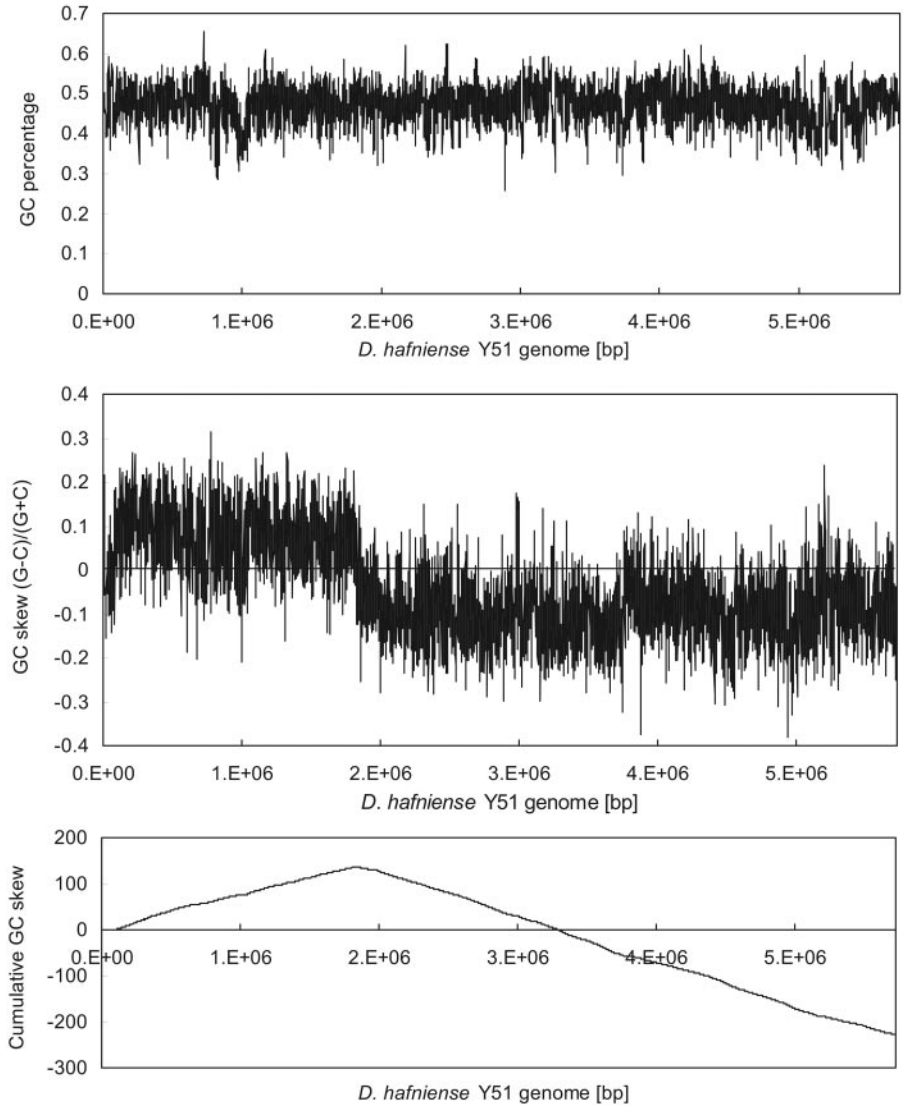

FIG. 2. G+C content, GC skew, and cumulative GC skew of the *D. hafniense* Y51 genome as determined using a 1,000-bp window.

some to date. The G+C content is 47.4%, and the overall variation of the G+C content in the genome is low (Fig. 2). Local changes in the coding density and the clustered presence of phage-related genes were identified, suggesting that multiple prophages in various states of decay are present in the genome (data not shown).

The genome is predicted to include six rRNA operons and 59 tRNA genes. There are several codons which are not represented by cognate tRNAs, suggesting that the codon recognition by the tRNA is wobbly in this organism. Eighty percent of the 5,060 predicted CDSs are transcribed in the same direction as DNA replication. Preferential use of the leading strand for transcription is also found in *Clostridium perfringens* (44) and *Clostridium tetani*.

D. hafniense Y51 belongs to the clostridia based on rRNA sequence comparison-based taxonomy. Consistent with this, the CDS homology search revealed that most *D. hafniense* Y51 CDSs exhibited the highest levels of similarity to CDSs of clostridia, including *T. tengcongensis*, a gram-negative, anaerobic, thiosulfate- and sulfur-reducing organism (2), and various *Clostridium* strains (Fig. 3). The next most prevalent group was the bacilli, which are known to be closely related to clostridia (Fig. 3). A large proportion of the CDSs, however, had no obvious orthologs or paralogs in clostridia or bacilli and exhibited the highest levels of similarity to CDSs of phylogenetically distant strains, especially members of the γ-*Proteobacteria* and *Archaea*, suggesting that the *D. hafniense* Y51 genome may contain many genes acquired by horizontal transfer at some stage of its evolution.

Of the of 5,060 predicted CDSs, over 75% had BLASTP hits to the COG database (50) with an e-value less than 1e-4. Functional classification of the predicted proteome revealed 430 CDSs related to energy production and conversion (functional classification group C) (Table 2).

Halogenated compounds as electron acceptors. From the viewpoint of dehalorespiration the most noteworthy group of respiratory enzymes is the corrinoid-containing reductive de-

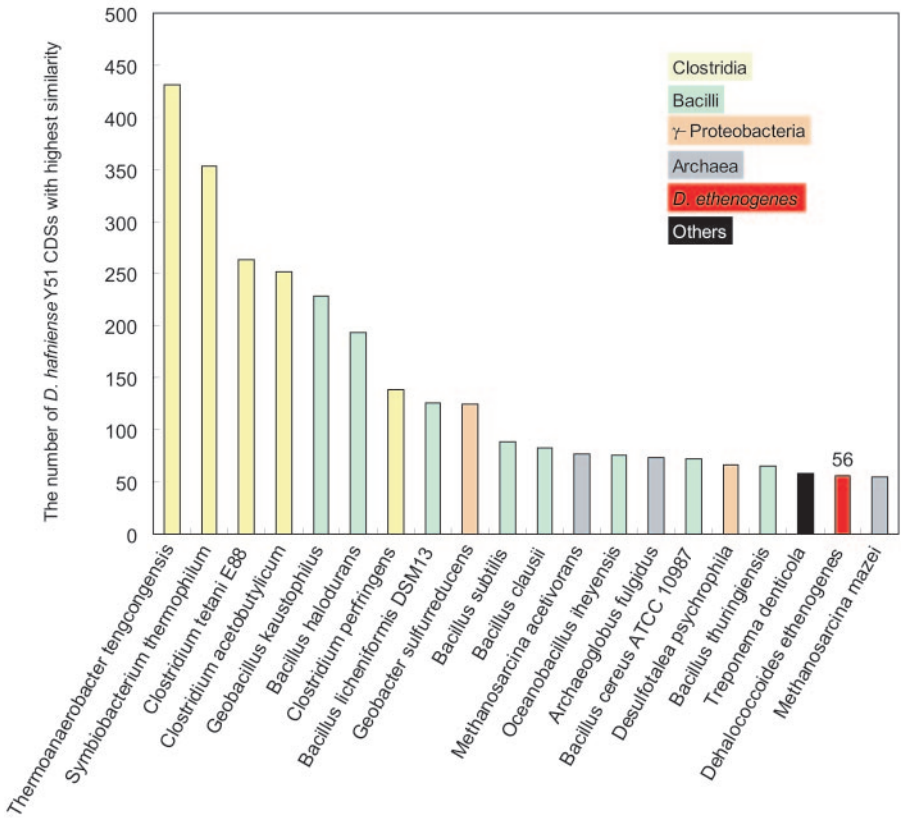

FIG. 3. Comparison of *D. hafniense* Y51 CDSs with CDSs of other organisms with completely sequenced genomes. Of more than 200 organisms in the database, the top 20 in terms of the number of highest-similarity hits to the 5,060 *D. hafniense* Y51 CDSs are shown. The taxonomic classifications of these 20 organisms are indicated by different colors.

halogenases (Fig. 4). The PCE dehalogenase encoded by *pceA* has been purified and characterized. It contains an Fe_4S_4 cluster binding motif and forms a complex with a membrane anchor subunit, PceB (49). A putative regulatory protein, PceC, and a trigger protein-like folding chaperone, PceT, are also encoded by the operon. A similar *pceABCT* cluster has also been reported in *Dehalobacter restrictus* and *D. hafniense* TCE1 (28). The cluster is sandwiched between the genes encoding two transposases in *D. hafninese* Y51, suggesting that it was acquired by horizontal transfer. PceA contains a Tat (twin arginine translocation) signal peptide (49) and is predicted to be transported through the cell membrane into the periplasmic space by the bacterial Tat-dependent type II secretion system as a prefolded complex (41). Four *tatA*-like genes and a *tatC*-like gene are present in the genome, but no *tatB* gene is present. This is unlike the situation in *Escherichia coli* (16), in which the type II secretion system was originally described, but it is just like the situation in *Bacillus subtilis* (18). In these microorganisms the TatA protein probably has a dual role and is also responsible for the TatB function. The other dehalogenase gene neither occurs in a cluster nor is surrounded by genes encoding transposases. The dehalogenase is very similar to the *ortho*-chlorophenol reductive dehalogenase of *Desulfitobacterium frappieri* PCP-1, which exhibits dechlorinating activity for several polychlorophenols (4). It is currently not known whether *D. hafniense* Y51 dechlorinates polychlorophenols. The finding that only two dehalogenase genes are present is a surprise considering that there are 19 such genes in the *D. ethenogenes* 195 genome (43) and nine such genes have been found in the partially sequenced strain *D. hafniense* DCB-2 (*D. hafniense* DCB-2 whole-genome shotgun project; GenBank accession number AAAW00000000). *D. hafniense* DCB-2 does not dechlorinate PCE and TCE, which may be explained by the presence of a different set of dehalogenases in this strain.

Electron acceptors other than halogenated compounds. In *D. hafniense* Y51 the CDSs that form the largest paralogous group are the CDSs that encode dimethyl sulfoxide (DMSO) reductase A subunits (*dmsA*) (3), most of which are accompanied by a CDS encoding small DmsB-like Fe-S cluster-containing accessory proteins (Tables 3 and 4). Many of the complexes are encoded by operons that also contain the genes for a DMSO reductase anchor subunit (*dmsC*) (54) or polysulfide reductase (*nrfD*) (17), two types of membrane subunits which are thought to participate in the electron transfer process (Table 4). These complexes are known to catalyze the reduction of DMSO, trimethylamine-*N*-oxide (40), arsenate (45), and a variety of other compounds, although the substrate specificities of most paralogs are not known. Indeed, these compounds can be utilized by this strain (data not shown).

Fumarate is the electron acceptor that leads to the fastest growth (48). It is predicted to be reduced by the three-subunit fumarate reductase encoded by *frdABC*. Interestingly, the genome encodes 30 paralogs of the flavoprotein subunit (*frdA*) (Tables 3 and 5) This group of coding sequences is also ex-

TABLE 2. COG-based functional categories of *D. hafniense* Y51 coding sequences

Category	Group	Functional classification (NCBI COG)	No. of CDSs[a]
Information storage and processing	J	Translation, ribosomal structure, and biogenesis	165
	K	Transcription	300
	L	DNA replication, recombination, and repair	222
Cellular processes	D	Cell division and chromosome partitioning	57
	O	Posttranslational modification, protein turnover, chaperones	86
	M	Cell envelope biogenesis, outer membrane	172
	N	Cell motility and secretion	132
	P	Inorganic ion transport and metabolism	209
	T	Signal transduction mechanisms	165
Metabolism	C	Energy production and conversion	430
	G	Carbohydrate transport and metabolism	102
	E	Amino acid transport and metabolism	255
	F	Nucleotide transport and metabolism	62
	H	Coenzyme metabolism	121
	I	Lipid metabolism	80
	Q	Secondary metabolite biosynthesis, transport, and catabolism	109
Multiple function			439
Poorly characterized	R	General function prediction only	392
	S	Unknown	275
No similarity to COGs with an e-value lower than 1e-4			1,287

[a] The total number of CDSs is 5,060.

panded in *Shewanella oneidensis* (Table 3). Nevertheless, the function of the flavoprotein subunits in fumarate reduction or other processes has not been established yet.

The genus *Desulfitobacterium* was originally described as a taxon containing organisms that reduce elemental sulfur and sulfite but not sulfate (51). *D. hafniense* Y51, however, has been reported to be capable of reducing sulfate (48). Indeed, the genome encodes sulfate reductases in addition to sulfite reductases (Table 5). *D. hafniense* Y51 also encodes a nitrate reductase, as well as two periplasmic nitrite reductase complexes (6) composed of a cytochrome *c* catalytic subunit, NrfA, and a cytochrome *c* membrane-anchoring subunit, NrfH (Table 5).

D. hafniense strains have been shown to be capable of utilizing metal ions as electron acceptors. The *D. hafnienese* Y51 genome encodes at least six *c*-type cytochromes, far fewer than the 111 and 42 paralogs found in metal ion-reducing strains of *Geobacter sulfurreducens* and *S. oneidensis*, respectively (31). Furthermore, tetraheme cytochrome *c* (*cymA*) required for *S. oneidensis* metal ion-dependent respiration (35, 42) is not present in *D. hafniense* Y51. This not only shows that the use of metal ions as electron acceptors may be rather limited but also hints that *c*-type cytochromes do not play a role in dehalorespiration.

Electron donors. *D. hafniense* Y51 cannot grow on mono- or oligosaccharides used as electron donors. We attribute this to the lack of suitable transport systems in this strain to import these compounds from the environment (functional classification G) (Table 2).

Both pyruvate and lactate have been reported to be used as electron donors by *D. hafniense* Y51 (48). Pyruvate is converted to acetate in the presence of PCE or TCE via a series of reactions (Fig. 5), as is the case in *Desulfitobacterium dehalogenans* (52). The *D. hafniense* Y51 genome encodes three pyruvate formate lyases and two pyruvate ferredoxin oxidoreductases that may mediate the conversion of pyruvate to acetyl coenzyme A (acetyl-CoA) (Fig. 5). Lactate may be oxidized to pyruvate by a broad-specificity malate dehydrogenase (27). The reducing equivalents formed during the conversion of pyruvate are channeled to the electron acceptors, including halogenated compounds (32). Growth on pyruvate or lactate is fast, since phosphate acetyltransferase and acetate kinases catalyze the conversion of acetyl-CoA to CoA and acetate coupled to substrate-level ATP synthesis; thus, not only energy is harnessed from the respiration process.

Formate supports slower growth than pyruvate or lactate, suggesting that there is no additional help from substrate-level phosphorylation when this electron donor is used. Formate might be metabolized by a selenocysteine-containing formate dehydrogenase (Table 5). Alternatively, formate might be split into hydrogen and carbon dioxide by the formate hydrogen lyase (Table 5), and the resulting hydrogen might be oxidized by a membrane-associated hydrogenase (Fig. 4). In contrast to the situation in *D. ethenogenes* 195, the use of hydrogen as an electron donor has not been proven to support *D. hafniense* Y51 growth. Nevertheless, three Hup-type Ni-Fe periplasmic hydrogenases and a putative Fe periplasmic hydrogenase, each with a cytochrome *b* subunit, and an Ni-Fe hydrogenase that is a neighbor of a putative cytochrome *c* similar to the split-soret cytochrome *c* of the sulfite-reducing organism *Desulfovibrio desulfuricans* (9) are encoded by *D. hafniense* Y51 (Table 5). Transposon-based mutagenesis of *D. dehalogenans* provided evidence for the involvement of a Hup-type hydrogenase and the formate hydrogen lyase-like complex in dehalorespiration (47); hence, the orthologous genes are likely to be essential components of

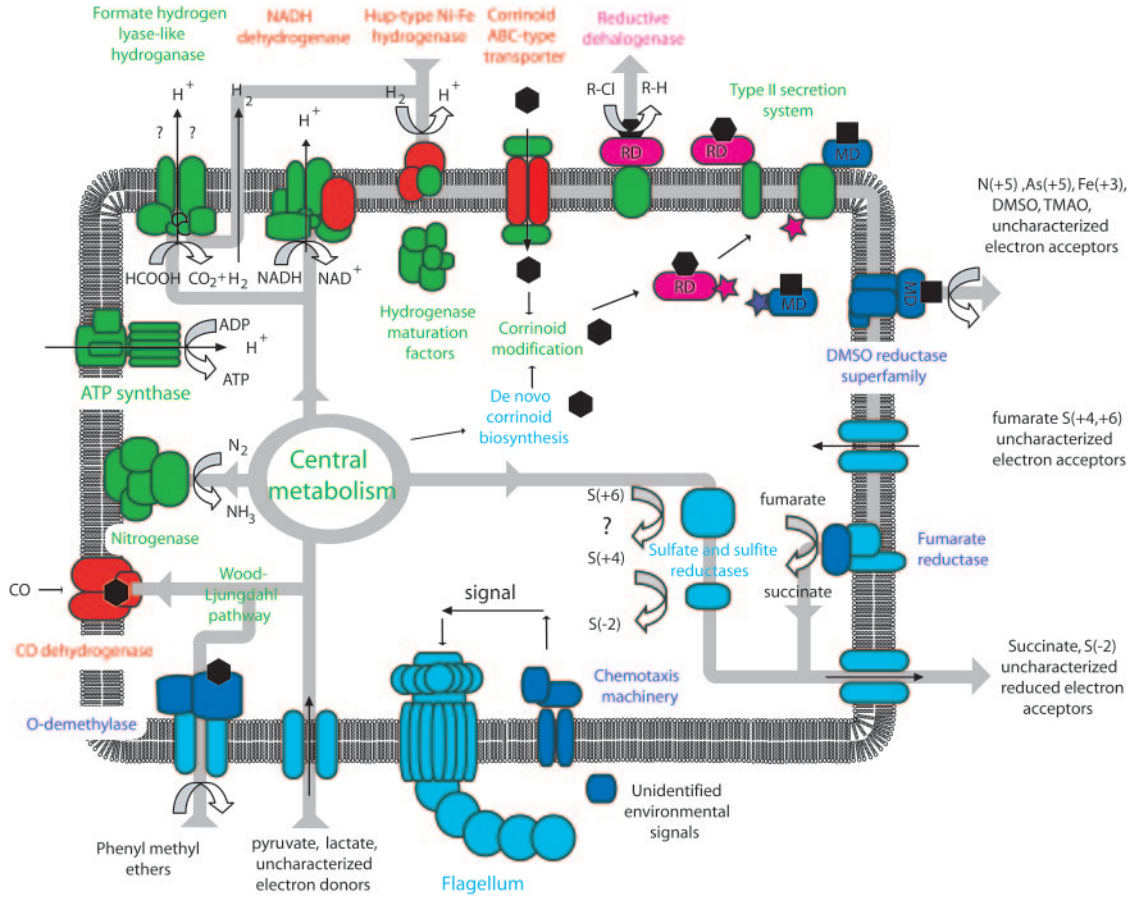

FIG. 4. Schematic diagram of the metabolic network in *D. hafniense* Y51 as determined by comparative analysis of the proteome with the proteomes of *D. ethenogenes* and other microorganisms. Note the versatility of the electron donors and electron acceptors that *D. hafniense* Y51 is predicted to utilize. The proposed routes of electron flow are indicated by wide gray lines. Modules that are shared by *D. hafniense* Y51 and *D. ethenogenes* but not predicted to play a role in dehalorespiration are green. Shared components that are envisaged to play an important role in reductive dehalogenation based on our comparative study are red. The reductive dehalogenase is purple. Modules that are present in *D. hafniense* Y51 but absent in *D. ethenogenes* are turquoise. Components that underwent a pronounced expansion in *D. hafniense* Y51 (Table 2) are blue. Black hexagons and squares represent corrinoid and molybdopterin cofactors, respectively. RD, reductive dehalogenase; MD, molybdopterin-containing DMSO reductase; TMAO, trimethylamine-*N*-oxide.

the genes encoding the dehalorespiration pathway. The genome also harbors the coding sequences for HypA to HypF implicated in hydrogenase maturation (26). The complex is encoded by the *hypAB* and *hypCDEF* operons in two different regions of the *D. hafniense* Y51 chromosome.

Many vanillate-specific *O*-demethylase corrinoid protein (*odmA*) (19) homologs support growth on lignin-derived compounds abundant in forest soil because phenyl methyl ethers are components of lignin in plants (20). *D. hafniense* PCE-S is known to utilize phenyl methyl ethers, including vanillate and syringate, as electron donors (36). *D. hafniense* Y51 contains 15 homologs of *odmA* (Table 3) and two genes similar to the vanillate:corrinoid protein methyltransferase gene (*odmB*) (19), suggesting that the use of phenyl methyl ethers is widespread in this species. Although not known to be autotrophic, *D. hafniense* Y51 contains the Wood-Ljungdahl pathway (22, 23, 34). This pathway might be used to channel methyl groups from phenyl methyl ethers to the central metabolism (Fig. 5). The presence of multiple paralogs suggests that they are important in *Desulfitobacterium* biology.

Central metabolism, cofactors, and oxidative stress. *D. hafniense* Y51 encodes a functional Embden-Meyerhof-Parnas pathway. Like many strict anaerobes, *D. hafniense* Y51 lacks 2-oxoglutarate dehydrogenase of the tricarboxylic acid cycle. This organism is predicted to be self-sufficient for nucleotides and amino acids, although apparently it cannot efficiently degrade them (data not shown). The genome encodes complete pathways for synthesis of the cofactors flavin adenine dinucleotide, NAD, menaquinone, heme, and cobalamin (Table 5). The presence of the cobalamin synthesis pathway is especially noteworthy, since it is required by both the phenyl methyl ether-utilizing *O*-demethylases and the reductive deghalogenases (Fig. 4).

The predicted nitrogenase complex of *D. hafniense* Y51 exhibited the highest levels of similarity to the complexes found in some methanogens and photosynthetic nitrogen-fixing bacteria (38). Experimental verification of the nitrogen-fixing ability of this strain is required for a more thorough understanding of these genes.

Desulfitobacteria have been characterized as organisms that grow only in strictly anaerobic conditions. The genomes of

TABLE 3. Selected clusters of orthologous groups with pronounced expansion in *D. hafniense* Y51 as judged by the number of paralogs in the genome compared to the genomes of nine completely sequenced organisms

Gene family as defined by COG	No. of paralogs[a]								
	Desulfitobacterium hafniense Y51 (5.7×10^6)[b]	*Clostridium acetobutylicum* ATCC 824 (3.9×10^6)	*Bacillus subtilis* subsp. *subtilis* strain 168 (4.2×10^6)	*Dehalococcoides ethenogenes* 195 (1.5×10^6)	*Desulfovibrio vulgaris* subsp. *vulgaris* strain Hildenborough (3.6×10^6)	*Geobacter sulfurreducens* PCA (3.8×10^6)	*Shewanella oneidensis* MR-1 (5.0×10^6)	*Escherichia coli* K-12 strain MG1655 (4.6×10^6)	*Archaeoglobus fulgidus* DSM4304 (2.2×10^6)
DMSO reductase superfamily and associated proteins									
Molybdopterin binding catalytic subunit (COG0243)	59	0	4	2	6	2	9	13	5
Fc-S cluster-containing small subunit (COG0437)	55	0	0	2	10	4	7	7	6
Membrane component type A (COG3302; *dmsC*)	17	0	0	0	0	0	0	2	0
Membrane component type B (COG3301; *nrfD*)	16	0	0	2	2	2	3	1	2
Uncharacterized component (COG3381)	16	0	0	0	0	0	4	3	0
Components of oxidoreductases									
Fumarate reductase, flavoprotein subunit (COG1053)	31	1	1	0	3	1	10	2	3
Fe-S cluster-containing oxidoreductases (COG0247)	15	0	3	0	9	4	1	3	12
Electron transfer flavoprotein alpha-subunit (COG2025)	9	2	1	0	0	1	1	3	1
Electron transfer flavoprotein beta-subunit (COG2086)	9	2	1	0	0	2	1	3	1
Cobalamin transport									
ABC-type cobalamin transporter (permease) (COG0609)	14	4	11	3	1	0	2	6	2
ABC-type cobalamin transporter (permease) (COG0614)	13	3	10	4	1	0	2	4	1
ABC-type cobalamin transporter (ATPase component) (COG1120)	11	3	5	5	2	0	2	4	2
Chemotaxis machinery									
Methyl-accepting chemotaxis protein (COG0840)	40	43	11	1	30	35	28	5	1
CheY-like receiver domain (COG0784)	8	7	5	0	15	24	9	2	9
Transcriptional regulators									
PAS, AAA-type ATPase and DNA-binding domain (COG3829)	29	2	2	0	3	1	0	1	0
Utilization of phenyl methyl ethers									
Cobalamin binding proteins, including *odmA* homologs (COG1410)	24	1	0	0	1	2	1	1	2

[a] The organisms belong to the following taxa: *Desulfitobacterium hafniense* Y51, *Firmicutes*, clostridia; *Clostridium acetobutylicum* ATCC 824, *Firmicutes*, clostridia; *Bacillus subtilis* subsp. *subtilis* strain 168, *Firmicutes*, bacilli; *Dehalococcoides ethenogenes* 195, *Chloroflexi*, *Dehalococcoidetes*; *Desulfovibrio vulgaris* subsp. *vulgaris* strain Hildenborough, *Proteobacteria*, *Deltaproteobacteria*; *Geobacter sulfurreducens* PCA, *Proteobacteria*, *Deltaproteobacteria*; *Shewanella oneidensis* MR-1, *Proteobacteria*, *Gammaproteobacteria*; *Escherichia coli* K-12 strain MG1655, *Proteobacteria*, *Gammaproteobacteria*; *Archaeoglobus fulgidus* DSM4304, *Euryarchaeota*, *Archaeoglobi*.

[b] The numbers in parentheses are genome sizes (in base pairs).

TABLE 4. Gene clusters encoding putative complexes of the DMSO reductase superfamily[a]

Cluster(s)	Type	CDSs[b]				Operon organization[c]
		COG0243 (A)	COG0437 (B)	COG3301 (N) or COG3302 (C)	COG3381 (T)	
1	I	DSY0186	DSY0187	DSY0188 (C)		ABC
2	IV	DSY0334				AOOO
3	I	DSY0357	DSY0356	DSY0355 (C)	DSY0358	TABC
4,5	II	DSY0526, DSY0527	DSY0528, DSY0531	DSY0529 (N)	DSY0530	AABNTB
6	II	DSY0547 (U)[d]	DSY0549	DSY0550 (N)		AOBN
7,8	III	DSY0586, DSY0590	DSY0585, DSY0587			AOOBAB
9	I	DSY0597	DSY0598	DSY0599 (C)	DSY0596	TABC
10	III	DSY0600	DSY0601			AB
11	III	DSY0777	DSY0778			AB
12	II	DSY0886	DSY0887	DSY0888 (N)	DSY0889	ABNT
13	II	DSY0893	DSY0894	DSY0895 (N)	DSY0896	ABNT
14	I	DSY1240	DSY1241	DSY1242 (C)		ABC
15	I	DSY1251	DSY1250	DSY1249 (C)		ABC
16	III	DSY1253	DSY1254			AB
17	III	DSY1266	DSY1267			AB
18	III	DSY1276	DSY1275			AB
19	III	DSY1277	DSY1278			AB
20	III	DSY1395	DSY1396			AB
21	I	DSY1448	DSY1449	DSY1450 (C)		ABC
22	IV	DSY1516				A
23	III	DSY1990	DSY1989			OBOAB
24	I	DSY2332	DSY2331	DSY2330 (C)		ABC
25	III	DSY2741	DSY2742			AB
26	II	DSY3058	DSY3057	DSY3056 (N)		ABN
27	II	DSY3062	DSY3061	DSY3060 (N)		ABN
28	IV	DSY3101 (U)				AOOO
29	I	DSY3410	DSY3409	DSY3408 (C)	DSY3411	TABC
30	III	DSY3413	DSY3412			AB
31	I	DSY3466	DSY3467	DSY3468 (C)		ABC
32	I	DSY3470	DSY3471	DSY3472 (C)		ABC
33	III	DSY3512	DSY3511			AB
34	III	DSY3518	(DSY3519)			AB
35	II	DSY3522	DSY3521	DSY3520 (N)		ABN
36	III	DSY3526	DSY3525			AB
37	III	DSY3535	DSY3534			AB
38	II	DSY3720	DSY3719	DSY3717 (N)	DSY3718	ABTN
39	II	DSY3724	DSY3723	DSY3722 (N)	DSY3721	ABNT
40	III	DSY3725	DSY3726			AB
41	I	DSY3752	DSY3751	DSY3750 (C)		ABC
42	II	DSY3896	DSY3895	DSY3894 (N)		ABN
43	IV	DSY3968				OOOA
44	IV	DSY4014				A
45	IV	DSY4078				A
46	I	DSY4087	DSY4086	DSY4085 (C)		ABC
47	I	DSY4090	DSY4089	DSY4088 (C)		ABC
48	II	DSY4131	DSY4132	DSY4130 (N)	DSY4133	NABT
49	III	DSY4603	DSY4602		DSY4601	ABT
50	III	DSY4610	DSY4609		DSY4608	ABT
51	II	DSY4615	DSY4617	DSY4616 (N)	DSY4614	BNAT
52	III	DSY4717	DSY4716			AB
53	III	DSY4765	(DSY4764)			AB
54	III	DSY4783	DSY4782			AB
55,56	I	DSY4820, DSY4821	DSY4819	DSY4818 (C)		ABC
57	I	DSY4824	DSY4823	DSY4822 (C)		ABC
58	II	DSY4906	DSY4904	DSY4903 (N)		AOBN
59	IV	DSY5019				A

[a] The gene clusters can be classified into four distinct groups based on the components encoded. Whereas type IV gene clusters contain only a single CDS encoding a COG0243 member, type III gene clusters also encode a COG0437 protein. In addition to COG0243 and COG0437, type I and II clusters encode membrane components of the COG03302 and COG03301 families, respectively.

[b] DSY0334 to DSY0337 encode a nitrate reductase (see Table 5), DSY3098 to DSY3101 encode a formate dehydrogenase (see Table 5), and DSY3968 to DSY3971 encode a putative hydrogenase (DSY3969 to DSY3971, *nuoGFE*).

[c] A, B, C, N, T, and O are CDSs encoding COG0243, COG0437, COG3302, COG3301, COG3381, and other functions, respectively.

[d] U indicates selenocysteine incorporation. *D. hafniense* Y51 can synthesize and incorporate selenocysteine into proteins, and its selenoproteome is predicted to consist of two proteins, both of which are members of the COG0243 group.

[e] DSY4820 and DSY4821 may be one protein.

TABLE 5. Selected protein coding sequences predicted to play an important physiological role in *D. hafniense* Y51 based on comparative studies with *D. ethenogenes* 195 and other microorganisms

Locus	Designation	Predicted function	Predicted localization	Orthologous CDS in *D. ethenogenes*
Predicted dehalogenases and related CDSs				
DSY1155		Probable chlorophenol reductive dehalogenase	Unknown	DET1070
DSY2834		Transposase	Cytoplasm	
DSY2836	*pceT*	Trigger factor	Cytoplasm	
DSY2837	*pceC*	Putative regulatory protein	Membrane	
DSY2838	*pceB*	Tetrachloroethene dehalogenase membrane-bound subunit	Membrane	
DSY2839	*pceA*	Tetrachloroethene dehalogenase	Periplasm[a]	DET0180
DSY2840		Transposase	Cytoplasm	
Predicted ATP synthases				
DSY4911	*atpC*	ATP synthase F1, epsilon subunit	Cytoplasm	DET0565
DSY4912	*atpD*	ATP synthase F1, beta subunit	Cytoplasm	DET0564
DSY4913	*atpG*	ATP synthase F1, gamma subunit	Cytoplasm	DET0563
DSY4914	*atpA*	ATP synthase F1, alpha subunit	Cytoplasm	DET0562
DSY4915	*atpH*	ATP synthase F1, delta subunit	Cytoplasm	DET0561
DSY4916	*atpF*	ATP synthase F0, B subunit	Cytoplasm	DET0560
DSY4917	*atpE*	ATP synthase F0, C subunit	Membrane	DET0559
DSY4918	*atpB*	ATP synthase F0, A subunit	Membrane	DET0558
Predicted CDSs involved in hydrogen metabolism, including NADH dehydrogenase				
DSY0794		Hup-type Ni,Fe hydrogenase cytochrome *b* subunit	Membrane	
DSY0795		Hup-type Ni,Fe hydrogenase large subunit	Unknown	
DSY0796		Hup-type Ni,Fe hydrogenase small subunit	Periplasm	DET0111
DSY1596		Ni,Fe-hydrogenase maturation factor	Cytoplasm	DET0109
DSY1597		Hup-type Ni,Fe hydrogenase cytochrome *b* subunit	Membrane	
DSY1598		Hup-type Ni,Fe hydrogenase large subunit	Periplasm	DET0110
DSY1599		Hup-type Ni,Fe hydrogenase small subunit	Periplasm	
DSY2100		Ni,Fe hydrogenase large subunit	Periplasm	
DSY2101		Ni,Fe hydrogenase small subunit	Periplasm	
DSY2238		Hup-type Ni,Fe hydrogenase small subunit	Periplasm	
DSY2239		Hup-type Ni,Fe hydrogenase large subunit	Periplasm	
DSY2240		Hup-type Ni,Fe hydrogenase cytochrome *b* subunit	Membrane	
DSY2474	*hypE*	Hydrogenase expression/formation protein HypE	Cytoplasm	DET1435
DSY2475	*hypD*	Hydrogenase expression/formation protein HypD	Cytoplasm	DET1434
DSY2476	*hypC*	Hydrogenase expression/formation protein HypC	Cytoplasm	DET1433
DSY2477	*hypF*	Hydrogenase maturation protein HypF	Cytoplasm	DET1432
DSY2578	*nuoN*	NADH dehydrogenase I chain N	Membrane	DET0933
DSY2579	*nuoM*	NADH dehydrogenase I chain M	Membrane	DET0932
DSY2580	*nuoL*	NADH dehydrogenase I chain L	Membrane	DET0931
DSY2581	*nuoK*	NADH dehydrogenase I chain K	Membrane	DET0930
DSY2582	*nuoJ*	NADH dehydrogenase I chain J	Membrane	DET0929
DSY2583	*nuoI*	NADH dehydrogenase I chain I	Unknown	DET0928
DSY2584	*nuoH*	NADH dehydrogenase I chain H	Membrane	DET0927
DSY2585	*nuoD*	NADH dehydrogenase I chain D	Cytoplasm	DET0926
DSY2586	*nuoC*	NADH dehydrogenase I chain C	Cytoplasm	DET0925
DSY2587	*nuoB*	NADH dehydrogenase I chain B	Unknown	DET0924
DSY2588	*nuoA*	NADH dehydrogenase I chain A	Membrane	DET0923
DSY3114	*hycG*	Formate hydrogen lyase subunit 7	Cytoplasm	DET1570
DSY3115	*hycE*	Formate hydrogen lyase subunit 5 precursor	Cytoplasm	DET1571
DSY3116	*hyfF*	Hydrogenase 4 component F	Membrane	DET1572
DSY3117	*hyfE*	Hydrogenase 4 component E	Membrane	DET1573
DSY3118	*hycC*	Formate hydrogenlyase subunit 4	Membrane	DET1574
DSY3119	*hyfB*	Hydrogenase 4 component B	Membrane	DET1575
DSY4710		Putative Fe hydrogenase cytochrome *b* subunit	Membrane	DET0186
DSY4711		Putative Fe hydrogenase iron-sulfur subunit	Periplasm	DET0112
DSY4712	*hydA*	Putative Fe hydrogenase large subunit	Periplasm	DET0147
DSY5043	*hypB*	Putative hydrogenase accessory protein HypB	Cytoplasm	DET1431
DSY5044	*hypA*	Putative hydrogenase nickel insertion protein HypA	Cytoplasm	DET1430
CDSs involved in biosynthesis of cobalamin				
DSY0318	*cobB*/*cbiA*	Cobyrinic acid a,c-diamide synthase	Unknown	

Continued on following page

TABLE 5—*Continued*

Locus	Designation	Predicted function	Predicted localization	Orthologous CDS in *D. ethenogenes*
DSY1479	*cbiM*	Cobalamin biosynthesis protein CbiM	Membrane	
DSY1561	*cobA*	Cob(I)alamin adenosyltransferase	Cytoplasm	
DSY1853	*cobA*	Cob(I)alamin adenosyltransferase	Cytoplasm	DET1224
DSY1979	*cobC*	Alpha-ribazole-5-phosphate phosphatase	Unknown	DET0693
DSY2114	*cobT*	Nicotinate-nucleotide-dimethylbenzimidazole phosphoribosyltransferase	Cytoplasm	DET0691
DSY2115	*cobU*	Cobinamide kinase/cobinamide phosphate guanylyltransferase	Cytoplasm	DET0694
DSY2116	*cobS*	Cobalamin (5′-phosphate) synthase	Membrane	DET0692
DSY2227	*cysG*	Putative precorrin-2 oxidase	Cytoplasm	
DSY3871	*cobN*	Cobalamin biosynthesis protein	Cytoplasm	
DSY3879	*cobA*	Cob(I)alamin adenosyltransferase	Cytoplasm	
DSY4059	*cobA*	Cob(I)alamin adenosyltransferase	Cytoplasm	
DSY4060	*cobB*/*cbiA*	Cobyrinic acid a,c-diamide synthase	Cytoplasm	DET0128
DSY4061	*cobD*	Cobalamin biosynthesis protein	Membrane	DET0688
DSY4062	*cobQ*	Cobyric acid synthase	Cytoplasm	DET0936
DSY4063	*cobH*/*cbiC*	Precorrin isomerase	Cytoplasm	
DSY4064	*cbiX*	Putative CbiX protein	Cytoplasm	
DSY4065	*cobK*/*cbiJ*	Cobalt-precorrin-6A reductase	Cytoplasm	
DSY4066	*cobJ*/*cbiH*	Cobalt-precorrin-3B C(17)-methyltransferase	Unknown	
DSY4067	*cbiG*	Cobalamin biosynthesis protein CbiG	Cytoplasm	
DSY4068	*cobM*/*cbiF*	Cobalt-precorrin-4 C(11)-methyltransferase	Cytoplasm	
DSY4070	*cobI*/*cbiL*	Cobalt-precorrin-2 C(20)-methyltransferase	Cytoplasm	
DSY4071	*cobL*/*cbiT*	Precorrin-6Y C(5,15)-methyltransferase (decarboxylating)	Cytoplasm	DET0296
DSY4072	*cbiD*	Cobalt-precorrin-6A synthase [deacetylating]	Unknown	
Well-characterized oxidoreductases				
DSY0309	*dsrA*	Sulfite reductase, dissimilatory-type alpha subunit	Cytoplasm	
DSY0310	*dsrB*	Sulfite reductase, dissimilatory-type beta subunit	Unknown	
DSY1869		Sulfite reductase, assimilatory type	Cytoplasm	
DSY2951	*cysN*	Adenylylsulfate kinase/sulfate adenylyltransferase subunit 1	Cytoplasm	
DSY2952	*cysD*	Sulfate adenylyltransferase subunit 2	Cytoplasm	
DSY2953	*cysH*	Phosphoadenosine phosphosulfate reductase	Unknown	
DSY0332		Nitrate/nitrite transporter	Membrane	
DSY0333		Nitrate/nitrite transporter	Membrane	
DSY0334	*narG*	Nitrate reductase alpha chain	Periplasm	
DSY0335	*narH*	Nitrate reductase beta chain	Periplasm	
DSY0336	*narJ*	Nitrate reductase delta chain	Unknown	
DSY0337	*narI*	Nitrate reductase gamma chain	Membrane	
DSY2471	*nrfH*	Cytochrome *c* nitrite reductase, small-subunit NrfH	Unknown	
DSY2472	*nrfA*	Cytochrome *c* nitrite reductase, catalytic subunit NfrA	Periplasm	
DSY3065	*nrfA*	Cytochrome *c* nitrite reductase, catalytic subunit NfrA	Periplasm	
DSY3066	*nrfH*	Cytochrome *c* nitrite reductase, small-subunit NrfH	Unknown	
DSY0735	*frdC*	Fumarate reductase cytochrome *b* subunit	Membrane	
DSY0736	*frdA*	Fumarate reductase flavoprotein subunit	Unknown	
DSY0737	*frdB*	Fumarate reductase iron-sulfur protein	Unknown	
DSY3098	*fdhE*	Putative formate dehydrogenase formation protein FdhE	Unknown	DET0185
DSY3099	*fdoI*	Formate dehydrogenase gamma subunit, cytochrome b_{556} subunit	Membrane	
DSY3100	*fdnH*	Formate dehydrogenase beta subunit, iron-sulfur subunit	Membrane	
DSY3101	*fdnG*	Formate dehydrogenase alpha subunit	Periplasm	DET0187

[a] Experimentally observed (49).

these bacteria encode five putative catalases, two superoxide dismutases, and several rubrerythrin-rubredoxin systems with four rubrerythrin and two rubredoxin paralogs. *D. hafniense* Y51 also has a cytochrome *bd* oxidase operon composed of four genes, the structure of which is similar to that of *Moorella thermoacetica* (8). The presence of these CDSs contributes to the relatively high tolerance of this strain to dioxygen.

Comparison of the *Desulfitobacterium and Dehalococcoides* genomes. It is very interesting that *D. hafniense* Y51 and *D. ethenogenes* 195 both have dechlorinating ability despite the fact that they are phylogenetically distantly related and have very different genomic features (Table 1). Since closely related *Desulfitobacterium* and *Dehalococcoides* strains contain vastly different numbers and kinds of dehalogenases, it is tempting to speculate that the genes are horizontally acquired due to anthropogenic environmental pressure (47).

To compare *D. hafniense* Y51 and *D. ethenogenes* 195, we identified an orthologous subset consisting of 751 genes in the two strains (Fig. 6), only 54 of which are related to energy production and conversion (Table 2). We argue that this set of

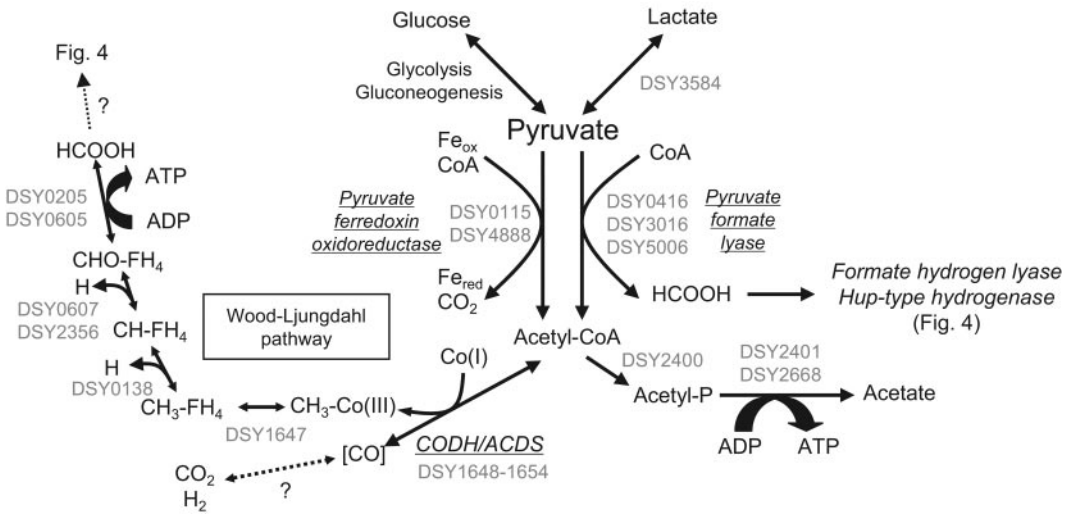

FIG. 5. Overview of part of the central metabolism of *D. hafniense* Y51 as predicted by the genome analysis.

751 coding sequences contains two classes, sequences that are responsible for dehalorespiration and sequences needed for other functions. While the former class is likely to be horizontally transferred (47), the latter class is predominantly vertically inherited and thus predicted to exhibit higher levels of homology to the orthologs of closely related strains than to the orthologs of strains which are more distantly related. By enriching for possible horizontally transferred genes within this subset, we should also enrich for coding sequences that may have a role in dehalorespiration.

To this end we identified the closest homologs of each of the 751 CDSs in the orthologous group to a CDS present in 1 of more than 200 completely sequenced organisms. Thirty-eight of the 751 *D. hafniense* Y51 coding sequences exhibited the highest levels of similarity to their *D. ethenogenes* 195 orthologs, and 72 of the 751 *D. ethenogenes* 195 coding sequences exhibited the highest levels of similarity to their *D. hafniense* Y51 orthologs (Fig. 6). For these two groups of coding sequences, 18 were found to be reciprocal best hits, meaning that the *D. hafniense* Y51 and *D. ethenogenes* 195 orthologs showed more homology to each other than they showed to any other paralogous sequence from another strain (Table 6 and Fig. 6.).

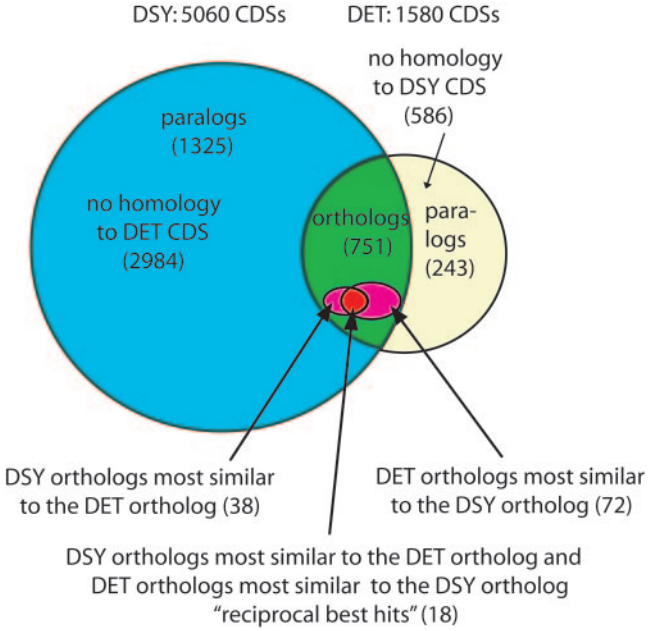

FIG. 6. Venn diagram comparing the coding sequence sets of *D. hafniense* Y51 (DSY) and *D. ethenogenes* 195 (DET) CDSs. The areas are proportional to the number of CDSs.

PceB and PceT have no obvious orthologs in *D. ethenogenes* 195, providing some circumstantial evidence that the membrane-anchoring mechanism is not conserved or dispensable and that the PceT trigger factor-like folding chaperone might not be essential or may be complemented with a nonhomologous protein. We, however, expected to find PceA and PceC among the reciprocal best hits. Surprisingly, PceA was not included in this group. Although our assumption was that *D. hafninese* Y51 and *D. ethenogenes* 195 were the only dehalorespirers among the microbial strains used for our comparative study, the genome of a recently sequenced marine microorganism, *Silicibacter pomeroyi* DSS-3 (33), contained a reductive dehalogenase gene. Nothing is known about the dehalorespiring capability of this microorganism, but the presence of a dehalogenase in yet another group of microorganisms provides additional evidence that the dehalogenases are frequently horizontally transferred. Exclusion of the coding sequences of this strain from the comparison did not modify any of our other results except that it added PceA as the 19th member of the reciprocal best-hit group (data not shown). As expected, the PceC-like putative transcription regulator was found in the group of 18 best hits, suggesting that there might be some similarity in the transcriptional regulation of the dehalogenases in the two organisms. Since there are many paralogs of this putative transcriptional factor in both organisms, our assumptions need to be corroborated experimentally.

Included in the group of 18 reciprocal best hits were the large subunit and the maturation factor of a Hup-type Ni-Fe

TABLE 6. Eighteen reciprocal best hits in the orthologous subset of 751 coding sequences

CDS in *D. hafniense*	Predicted function	CDS in *D. ethenogenes*
DSY0391	ABC-type cobalamin/Fe^{3+}-siderophore transport system, permease component	DET1175
DSY0393	ABC-type cobalamin/Fe^{3+}-siderophore transport system, unknown component	DET1173
DSY1228	Hypothetical protein	DET0516
DSY1247	Similar to COG0348 polyferredoxin	DET0237
DSY1596	Hup-type Ni,Fe-hydrogenase maturation factor	DET0109
DSY1598	Hup-type Ni,Fe-hydrogenase large subunit	DET0110
DSY1648	Carbon monoxide dehydrogenase/acetyl-CoA synthase delta subunit	DET0701
DSY1650	Carbon monoxide dehydrogenase/acetyl-CoA synthase ferredoxin-like protein	DET0704
DSY1651	Carbon monoxide dehydrogenase/acetyl-CoA synthase gamma subunit	DET0699
DSY1652	Carbon monoxide dehydrogenase/acetyl-CoA synthase alpha subunit	DET0700
DSY1671	Similar to COG0655 multimeric flavodoxin WrbA	DET1371
DSY1890	Similar to COG0489 ATPases involved in chromosome partitioning	DET0104
DSY2085	ABC-type cobalamin/Fe^{3+}-siderophore transport systems, permease component	DET0685
DSY2558	Hypothetical protein	DET0698
DSY2585	NADH dehydrogenase I chain D	DET0926
DSY3715	PccC like transcriptional regulator	DET1598
DSY4099	Hypothetical protein	DET1387
DSY4876	Hypothetical protein	DET0416

hydrogenase. Since it has been experimentally proven that Hup-type hydrogenases are necessary for dehalorespiration (47), the high level of similarity of the *D. hafniense* Y51 and *D. ethenogenes* 195 orthologs tempted us to speculate about the existence of a dehalorespiration-specific Ni-Fe hydrogenase. Both strains possess multiple copies of various corrinoid transport systems, multiple subunits of which exhibit unusually high levels of similarity. This clearly highlights the importance of scavenging corrinoid cofactors from the environment (Fig. 4). This is particularly important for *D. ethenogenes* 195, which, unlike *D. hafniense* Y51, does not encode the complete de novo corrinoid synthesis pathway (Table 3). Although carbon monoxide dehydrogenase activity is not known to be involved in dehalorespiration, the CDSs encoding the putative carbon monoxide dehydrogenase/acetyl-CoA synthase (Fig. 5) are conserved to a great extent in the two strains, as are several uncharacterized coding sequences which to date have not been implicated in dehalorespiration or other processes.

Although the similarity of *D. hafniense* Y51 and *D. ethenogenes* 195 is interesting from the viewpoint of dehalorespiration, the differences are also noteworthy. *D. hafniense* Y51 contains an unprecedented number and variety of respiration-related genes, most of which are not present in *D. ethenogenes* 195 (Tables 3 and 5). This should be one reason why *D. ethenogenes* 195 utilizes only hydrogen as an electron donor and chlorinated organic compounds as electron acceptors (29). *D. hafniense* Y51 is known to possess a flagellum and is highly motile (48). Indeed, the genome encodes multiple copies of methyl-accepting chemotaxis proteins (Table 3) and contains a large cluster of motility genes. It should be interesting to study whether chlorinated compounds act as chemoattractants for this strain. In contrast, *D. ethenogenes* 195 is a coccoid organism whose genome encodes no motility. This is a disadvantage in bioremediation studies, since this species might not be as efficient in locating and approaching the target to be degraded.

The comparison of the genomes of *D. hafniense* Y51 and *D. ethenogenes* 195 showed that two superficially similar organisms, both of which were isolated based on their PCE-reducing abilities, are very different. Although excelling in the variety of chlorinated compounds that it can use as electron acceptors, *D. ethenogenes* 195 is a true dechlorination specialist; its limited metabolic repertoire and its apparent inability to disperse efficiently in the environment probably mean that this organism must be used as part of a bacterial community in bioremediation. *D. hafniense* Y51, on the other hand, is a generalist. It exhibits very high and still unexplored flexibility and uses a wide variety of electron donors and acceptors, which broadens the scope of its biotechnological applications. It is motile and largely self-sufficient for factors needed for reductive dehalogenation. The two genomes not only establish a firm background for research on dehalorespiration but pave the way for metabolic engineering of these strains to better suit the purposes of bioremediation.

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