Genome analysis of *Desulfotomaculum kuznetsovii* strain 17T reveals a physiological similarity with *Pelotomaculum thermopropionicum* strain SIT

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Desulfotomaculum kuznetsovii is a moderately thermophilic member of the polyphyletic spore-forming genus *Desulfotomaculum* in the family *Peptococcaceae*. This species is of interest because it originates from deep subsurface thermal mineral water at a depth of about 3,000 m. *D. kuznetsovii* is a rather versatile bacterium as it can grow with a large variety of organic substrates, including short-chain and long-chain fatty acids, which are degraded completely to carbon dioxide coupled to the reduction of sulfate. It can grow methylotrophically with methanol and sulfate and autotrophically with $H_2 + CO_2$ and sulfate. For growth it does not require any vitamins. Here, we describe the features of *D. kuznetsovii* together with the genome sequence and annotation. The chromosome has 3,601,386 bp organized in one contig. A total of 3,567 candidate protein-encoding genes and 58 RNA genes were identified. Genes of the acetyl-CoA pathway, possibly involved in heterotrophic growth with acetate and methanol, and in CO_2 fixation during autotrophic growth are present. Genomic comparison revealed that *D. kuznetsovii* shows a high similarity with *Pelotomaculum thermopropionicum*. Genes involved in propionate metabolism of these two strains show a strong similarity. However, main differences are found in genes involved in the electron acceptor metabolism.

Introduction

Desulfotomaculum kuznetsovii strain 17T (VKM B-1805; DSM 6115) is a moderately thermophilic sulfate-reducing bacterium isolated from deep subsurface thermal mineral water [1]. It grows with a wide range of substrates, including organic acids, such as long-chain fatty acids, short-chain fatty acids (butyrate, propionate, acetate), lactate, pyruvate, fumarate and succinate as well as ethanol and methanol. These substrates are degraded to CO_2 coupled to sulfate reduction. The strain is also able to grow autotrophically with H_2/CO_2 and sulfate and to ferment pyruvate and fumarate. For growth, *D. kuznetsovii* has no vitamin requirement.

Desulfotomaculum is a genus of Gram-positive, spore-forming anaerobes that is phylogenetically and physiologically very diverse. The genus is poorly studied physiologically, while its members are known to play an important role in the carbon and sulfur cycle in a variety of often adverse environments. The genus is divided phylogenetically into different sub-groups [2,3]. To get a thorough understanding of the evolutionary relationship of the different *Desulfotomaculum* sub-groups and the physiology of the individual species, it is important to have genome sequence information. Here, we present a summary of the features of *D. kuznetsovii* strain 17T, together with the description of the complete genomic sequencing and annotation. Moreover, we describe a physiological and genomic comparison of *D. kuznetsovii* strain 17T and *Pelotomaculum thermopropionicum* strain SIT, because phylogenetically *P. thermopropionicum* is the closest related organism with validly published name that has a completely sequenced genome. However, the two strains have different physiological traits. For example, *P. thermopropionicum* is not able to grow by sulfate reduction, but is able to grow in syntrophy with methanogens. *D. kuznetsovii* lacks this ability. By comparing the genomes of the two bacteria we were able to identify the main similarities and differences.

Classification and features

D. kuznetsovii is a member of the phylum *Firmicutes*. Phylogenetic analysis of the 16S rRNA genes of *D. kuznetsovii* shows that it clusters in *Desulfotomaculum* cluster 1. This cluster not only contains *Desulfotomaculum* species, but also members of the genera *Sporotomaculum*, *Cryptanaerobacter* and *Pelotomaculum*. *D. kuznetsovii* is part of sub-group 1c together with *D. solfataricum*, *D. luciae*, *D. thermosubterraneum*, *D. salinum*, *D. australicum*, and *D. thermocisternum*, while *Pelotomaculum* species belong to sub-group 1h (Figure 1) [2].

D. kuznetsovii cells are rod-shaped (1.0-1.4 x 3.5-5 μm) with rounded ends and peritrichous flagella [Figure 2]. Spores of *D. kuznetsovii* are spherical (1.3 μm in diameter) and centrally located causing swelling of the cells. *D. kuznetsovii* grows between 50 and 85°C, but the optimal growth temperature is 60-65°C. The substrates *D. kuznetsovii* can grow with are completely oxidized to CO_2. Suitable electron acceptors are sulfate, thiosulfate and sulfite.

D. kuznetsovii is also able to grow by fermentation of pyruvate and fumarate. A summary of the classification and general features of *D. kuznetsovii* is presented in Table 1 [1].

Genome sequencing and annotation
Genome project history
D. kuznetsovii was selected for sequencing in the DOE Joint Genome Institute Community Sequencing Program 2009, proposal 300132_795700 'Exploring the genetic and physiological diversity of *Desulfotomaculum* species', because of its phylogenetic position in one of the *Desulfotomaculum* sub-groups, its important role in bioremediation, and its ability to use propionate, acetate and methanol for growth. The genome project is listed in the Genome OnLine Database (GOLD) [20] as project Gc01781, and the complete genome sequence was deposited in Genbank. Sequencing, finishing and annotation of the *D. kuznetsovii* genome were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.

Growth conditions and DNA isolation
D. kuznetsovii was grown anaerobically at 60°C in bicarbonate buffered medium with propionate and sulfate as substrates [1]. DNA of cell pellets was isolated using the standard DOE-JGI CTAB method recommended by the DOE Joint Genome Institute (JGI, Walnut Creek, CA, USA). In short, cells were resuspended in TE (10 mM tris; 1 mM EDTA, pH 8.0). Subsequently, cells were lysed using lysozyme and proteinase K, and DNA was extracted and purified using CTAB and phenol:chloroform:isoamylalcohol extractions. After precipitation in 2-propanol and washing in 70% ethanol, the DNA was resuspended in TE containing RNase. Following a quality and quantity check using agarose gel electrophoresis in the presence of ethidium bromide, and spectrophotometric measurement using a NanoDrop ND-1000 spectrophotometer (NanoDrop® Technologies, Wilmington, DE, USA).

Genome sequencing and assembly
The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website [21]. Pyrosequencing reads were assembled using the Newbler assembler (Roche).

The initial Newbler assembly consisting of 81 contigs in five scaffolds was converted into a phrap [22] assembly by making fake reads from the consensus, to collect the read pairs in the 454 paired end library. Illumina GAii sequencing data (570.2 Mb) was assembled with Velvet [23] and the consensus sequences were shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. The 454 draft assembly was based on 134.6 Mb 454 draft data and all of the 454 paired end data. Newbler parameters are -consed -a 50 -l 350 -g -m -ml 20. The Phred/Phrap/Consed software package [22] was used for sequence assembly and quality assessment in the subsequent finishing process. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with gapResolution [21], Dupfinisher [24], or sequencing cloned bridging PCR fragments with subcloning. Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (J.-F. Chang, unpublished). A total of 400 additional reactions and one shatter library were necessary to close gaps and to raise the quality of the finished sequence. Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed at JGI [25]. The error rate of the completed genome sequence is less than 1 in 100,000. Together, the combination of the Illumina and 454 sequencing platforms provided 188.8 × coverage of the genome. The final assembly contained 323,815 pyrosequence and 15,594,144 Illumina reads.
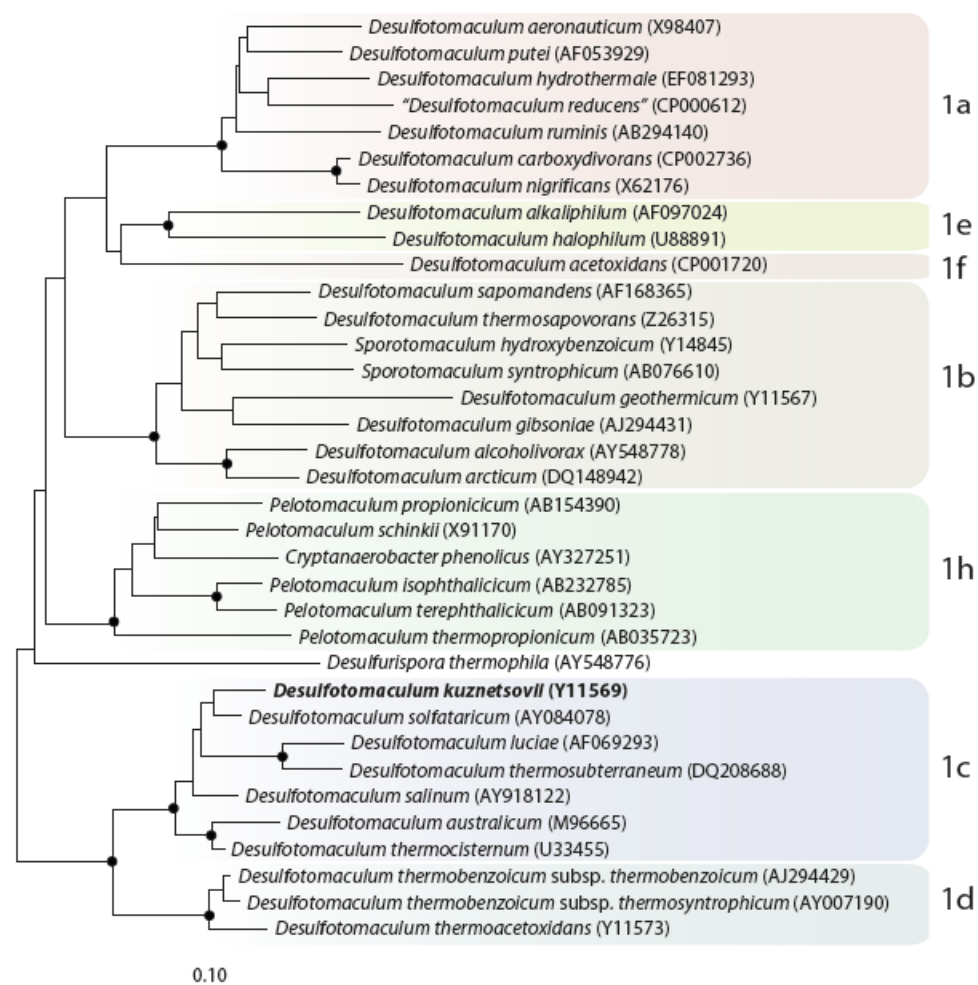

Figure 1. Neighbor joining tree based on 16S rRNA sequences showing the phylogenetic affiliation of *Desulfotomaculum* and related species divided in the subgroups of *Desulfotomaculum* cluster 1. *D. kuznetsovii* is printed in bold type. The sequences of different *Thermotogales* were used as outgroup, but were pruned from the tree. Closed circles represent bootstrap values between 75 and 100%. The scale bar represents 10% sequences difference.

Genome annotation

Genes were identified using Prodigal [26] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline [27]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes - Expert Review (IMG-ER) platform [28].

Genome properties and genome comparison with other strains

The genome of *D. kuznetsovii* consists of a circular chromosome of 3,601,386 bp with 54.88% GC content (Table 3 and Figure 3). Pseudogenes comprise 4.66% of the genes identified. Of the 3,625 genes predicted, 3,567 are protein-coding genes of which 2,560 are assigned to COG functional categories. The distribution of these genes into COG functional categories is presented in Table 4.

Table 1. Classification and general features of *D. kuznetsovii* DSM 6115 according to the MIGS recommendations [4].

MIGS ID	Property	Term	Evidence code[a]
		Domain *Bacteria*	TAS [5]
		Phylum *Firmicutes*	TAS [6-8]
		Class *Clostridia*	TAS [9,10]
	Current classification	Order *Clostridiales*	TAS [11,12]
		Family *Peptococcaceae*	TAS [11,13]
		Genus *Desulfotomaculum*	TAS [11,14,15]
		Species *Desulfotomaculum kuznetsovii*	TAS [1,16]
		Type strain 17	
	Gram stain	Positive	TAS [1]
	Cell shape	Rods	TAS [1]
	Motility	peritrichous flagella	TAS [1]
	Sporulation	oval, terminal or subterminal, slightly swelling the cell.	TAS [1]
	Temperature range	50-85°C	TAS [1]
	Optimum temperature	60-65°C	TAS [1]
	Carbon source	CO_2 (autotrophic) and organic substrates (heterotrophic)	TAS [1]
	Energy source	Sulfate-dependent growth and fermentative growth with pyruvate and fumarate.	TAS [1]
	Electron acceptor	Sulfate, thiosulfate and sulfite.	TAS [1]
MIGS-6	Habitat	Geothermal groundwater, sediment and hot solfataric fields.	TAS [1,17,18]
MIGS-6.3	Salinity	2-3% NaCl	TAS [1]
MIGS-22	Oxygen	Obligate anaerobes	TAS [1]
MIGS-15	Biotic relationship	Free living	TAS [1]
MIGS-14	Pathogenicity	None	
MIGS-4	Geographic location	Sukhumi, Georgia	TAS [1]
MIGS-5	Sample collection time	1987 or before	TAS [1]
MIGS-4.1	Latitude	43.009	TAS [1]
MIGS-4.2	Longitude	40.989	TAS [1]
MIGS-4.3	Depth	2800-3250 m	TAS [1]

Evidence codes - TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). Evidence codes are from the Gene Ontology project [19].
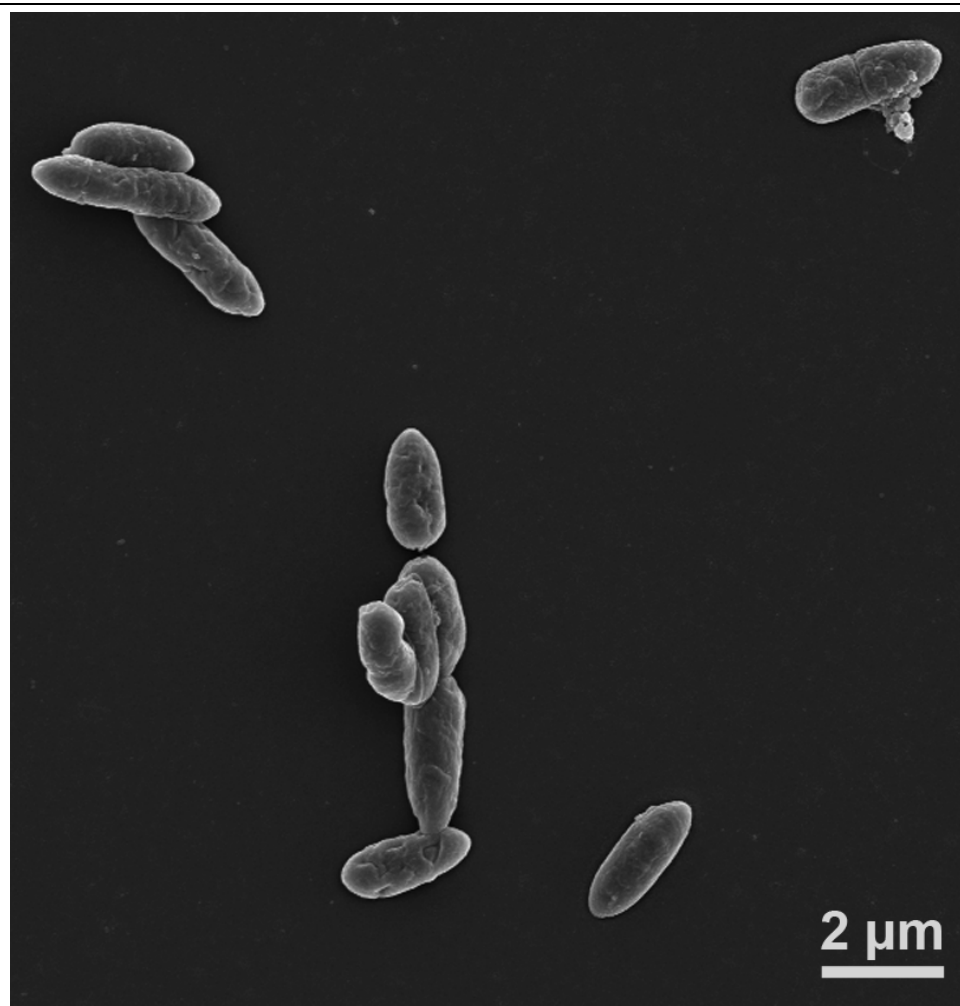
Figure 2. Scanning electron microscopic photograph of *D. kuznetsovii*.

Table 2. Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Finished
MIGS-28	Libraries used	Four genomic libraries: one Illumina shotgun library, one 454 standard library, two paired end 454 libraries
MIGS-29	Sequencing platforms	Illumina GAii, 454 Titanium
MIGS-31.2	Fold coverage	158.2 × illumina; 30.6 × pyrosequencing
MIGS-30	Assemblers	VELVET version 0.7.63; Newbler version 2.3; phrap version SPS - 4.24
MIGS-32	Gene calling method	Prodigal 1.4, GenePRIMP
	INSDC ID	CP002770.1
	Genome Database release	July 20, 2012
	Genbank Date of Release	May 24, 2011
	GOLD ID	Gc01781
	NCBI project ID	48313
MIGS-13	Source material identifier	DSM 6115^T
	Project relevance	Obtain insight into the phylogenetic and physiological diversity of *Desulfotomacum* species, and bioremediation.

Table 3. Genome statistics

Attribute	Value	% of total[a]
Genome size (bp)	3,601,386	100.00
Genome coding region (bp)	3,057,959	84.91
Genome G+C content (bp)	1,976,601	54.88
Total genes	3,625	100.00
RNA genes	58	1.60
Protein-coding genes	3,567	98.40
Genes in paralog clusters	1,373	37.88
Genes assigned to COGs	2,560	70.62
Pseudo genes	169	4.66
Genes with signal peptides	582	16.06
Genes with transmembrane helices	748	20.63
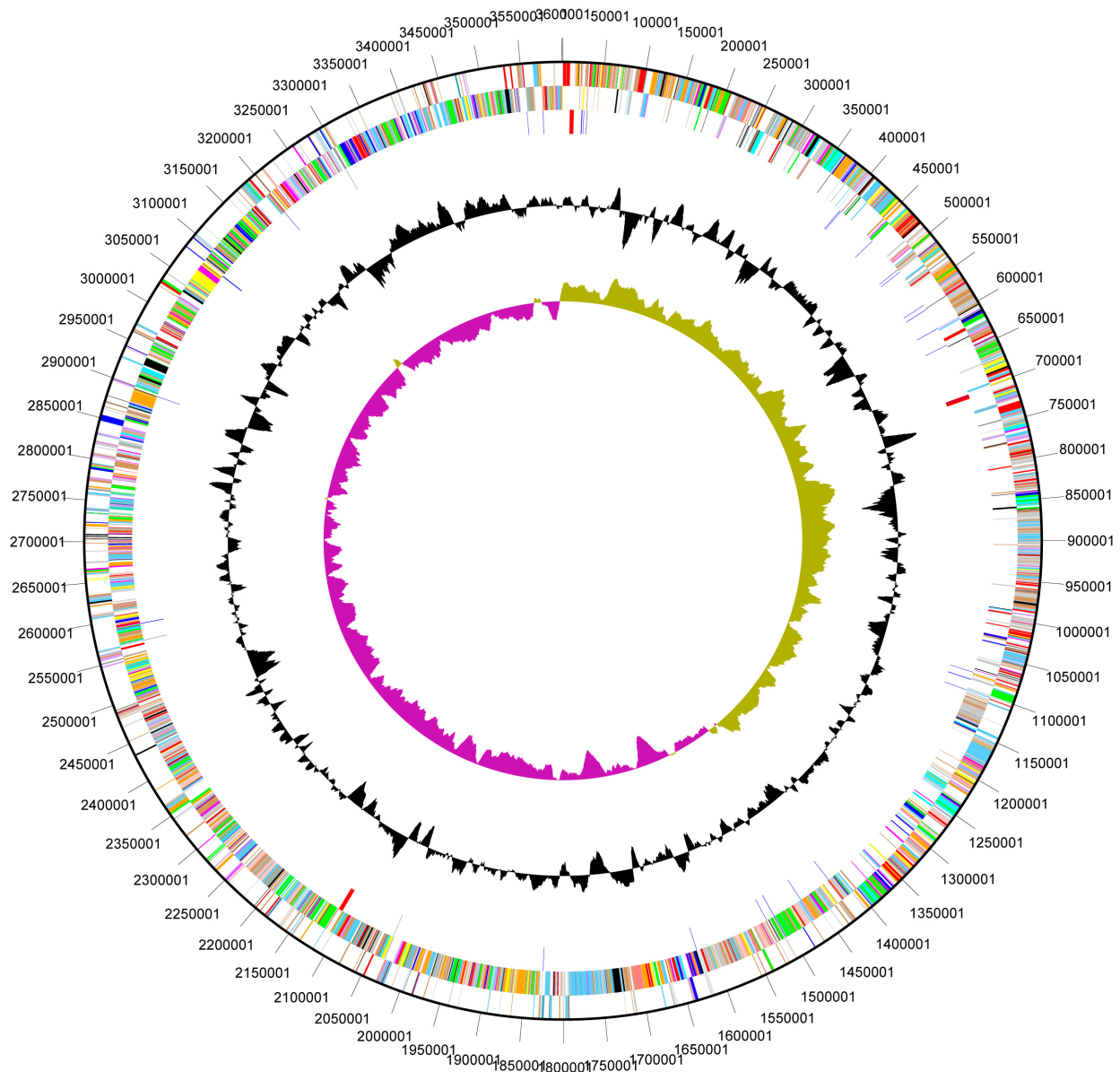
Figure 3. Graphical map of the chromosome of *D. kuznetsovii*. From outside to the center: Genes on the forward strand (colored by COG categories), Genes on the reverse strand (colored by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.

The genome of *D. kuznetsovii* has 58 RNA genes of which, three are 16S rRNA genes. This is one more than the previously described *rrnA* and *rrnB* [29]. These two rRNA genes contained two large inserts. One at the variable 5'terminal region and one at the variable 3'terminal region. The main differences between the two rRNA genes were found in these inserts. These inserts were hypothesized to be involved in the operation of ribosomes at high temperatures. However, more research is needed to assess the function of these inserts. All three rRNA genes of *D. kuznetsovii* have a size of approximately 1,700 nucleotides. This suggests that the third rRNA gene might also contain inserts. Alignment of the 16S rRNA genes confirmed the presence of inserts in all three 16S rRNA genes (data not shown).

BLAST analysis [30,31] of the genes of *D. kuznetsovii* against genes in the KEGG Sequence Similarity DataBase revealed similarity with other *Desulfotomaculum* strains (Table 5), *D. acetoxidans*, *D. carboxydivorans*, *"D. reducens"* and *D. ruminis*, but interestingly also with non-*Desulfotomaculum* strains. *D. kuznetsovii* contains 873 genes with high similarity to genes of *Pelotomaculum thermopropionicum*, which is more than to any of the sequenced *Desulfotomaculum* species. Moreover, we identified the conserved proteins of *D. kuznetsovii* across three related fully sequenced species (Table 6). The bidirectional best blast hits showed that despite the smaller genome of *P. thermopropionicum* it contained more homologous predicted proteins with *D. kuznetsovii* (1,406) compared to *D. acetoxidans* (1,309) and *"D. reducens"* (1330). This suggests a strong physiological similarity between *D. kuznetsovii* and *P. thermopropionicum*.

Table 4. Number of genes associated with the general COG functional categories

Code	Value	%age[a]	Description
J	148	5.32	Translation
A	0	0.00	RNA processing and modification
K	184	6.61	Transcription
L	207	7.44	Replication, recombination and repair
B	2	0.07	Chromatin structure and dynamics
D	60	2.16	Cell cycle control, mitosis and meiosis
Y	0	0.00	Nuclear structure
V	35	1.26	Defense mechanisms
T	177	6.36	Signal transduction mechanisms
M	122	4.38	Cell wall/membrane biogenesis
N	79	2.84	Cell motility
Z	2	0.07	Cytoskeleton
W	0	0.00	Extracellular structures
U	75	2.69	Intracellular trafficking and secretion
O	81	2.91	Posttranslational modification, protein turnover, chaperones
C	261	9.38	Energy production and conversion
G	106	3.81	Carbohydrate transport and metabolism
E	197	7.08	Amino acid transport and metabolism
F	55	1.98	Nucleotide transport and metabolism
H	158	5.68	Coenzyme transport and metabolism
I	89	3.20	Lipid transport and metabolism
P	127	4.56	Inorganic ion transport and metabolism
Q	122	3.58	Secondary metabolites biosynthesis, transport and catabolism
R	331	11.89	General function prediction only
S	257	9.23	Function unknown
-	1,065	29.38	Not in COGs

Table 5. Taxonomic distribution of the top KEGG hits of *D. kuznetsovii* genes based on BLAST against KEGG database.[†]

Kingdom	Category	Species	Hits
Archaea			91
	Crenarchaeota		9
	Euryarchaeota		81
	Thaumarchaeota		1
Bacteria			2,963
	Acidobacteria		2
	Actinobacteria		16
	Alphaproteobacteria		13
	Bacteroidetes		5
	Betaproteobacteria		14
	Cyanobacteria		19
	Deinococcus-Thermus		16
	Deltaproteobacteria		62
	Epsilonproteobacteria		1
	Firmicutes		2,728
		Ammonifex degensii	170
		Carboxydothermus hydrogenoformans	58
		Desulfotomaculum acetoxidans	310
		Candidatus Desulforudis audaxviator	154
		Desulfotomaculum carboxydivorans	268
		Desulfotomaculum reducens	111
		Desulfotomaculum ruminis	132
		Moorella thermoacetica	183
		Pelotomaculum thermopropionicum	873
		Thermincola potens JR	104
	Fusobacteria		2
	Gammaproteobacteria		12
	Green nonsulfur bacteria		20
	Green sulfur bacteria		4
	Hyperthermophilic bacteria		31
	Other *Proteobacteria*		1
	Spirochaetes		9
	Synergistetes		6
	Verrucomicrobia		2
Eukaryotes			3
	Plants		1
	Protists		2
Null			342
Total			3,399

[†]Species that had more than 50 genes similar to *D. kuznetsovii* were included in this table, others were only summarized in categories.

Insights into the genome
Involvement of the acetyl-coA pathway in growth with acetate and methanol

D. kuznetsovii oxidizes acetate completely to CO_2. The pathway of acetate degradation has not been studied yet, but sulfate reducers may employ the tricarboxylic acid (TCA) cycle or the acetyl-CoA pathway for acetate degradation, as exemplified by *Desulfobacter postgatei* and *Desulfobacca acetoxidans*, respectively [32]. Most genes predicted to code for enzymes of the TCA cycle are present in the genome of *D. kuznetsovii*, but genes with similarity to those coding for an ATP-dependent citrate synthase and isocitrate dehydrogenase are missing. This suggests that the TCA cycle is not complete and that the TCA cycle enzymes have mainly an anabolic function or a function in other catabolic pathways, such as the propionate degradation pathway. Genes with similarity to those coding for enzymes involved in the acetyl-CoA pathway are all present in the genome of *D. kuznetsovii* (Figure 4), which suggests its involvement in acetate oxidation. However, there are no genes similar to those that code for acetate kinase and phosphate acetyltransferase present in the genome. The reaction from acetate to acetyl-CoA is likely performed by acetyl-CoA synthetase (Desku_1241).

D. acetoxidans is an acetate-oxidizing *Desulfotomaculum* species, positioned in subgroup 1e (Figure 1), that also uses the acetyl-CoA pathway for acetate oxidation to CO_2 [33]. The genes involved in acetate oxidation in *D. acetoxidans* are similar to those in *D. kuznetsovii*, but there are some exceptions. The genome of *D. acetoxidans* does not contain a gene that putatively codes for acetyl-CoA synthetase, similar to *D. kuznetsovii*, but contains genes that putatively code for an acetate kinase and a phosphate acetyltransferase [34]. Additionally, putative carbon-monoxide dehydrogenase complex coding genes involved in the acetyl-CoA pathway show differences between the two *Desulfotomaculum* species. *D. kuznetsovii* lacks a ferredoxin coding gene that is located between *cooC* (Desku_1493) and *acsE* (Desku_1487), which in contrast is present in the genome of *D. acetoxidans* (Dtox_1273). Moreover, three genes similar to heterodisulfide reductase encoding genes (Desku_1486-1484) are located upstream of *acsE* in *D. kuznetsovii*, which is not the case in the genome of *D. acetoxidans.*

Table 6. Proteins of *D. kuznetsovii* conserved across three related species with fully sequenced genomes[†].

Subject DB Input Query	D. acetoxidans	D. kuznetsovii	D. reducens	P. thermopropionicum
D. acetoxidans	4,068	1,539	1,525	1,486
		1,309	1,316	1,255
D. kuznetsovii	1,509		1,518	1,645
	1,309	3,398	1,330	1,406
D. reducens	1,537	1,571		1,438
	1,316	1,330	3,276	1,211
P. thermopropionicum	1,430	1,600	1,395	
	1,255	1,406	1,211	2,919

[†] BLAST analyses were performed using standard settings and best hits were filtered for 40% identity over an alignment length of 75 amino acids as a minimum requirement. The values show the number of predicted proteins that are homologous to the query species in each row. The number of similar proteins obtained with a unidirectional BLAST is indicated in light blue. Bidirectional best blast hits are indicated in dark blue. Proteomes were obtained from ftp.ncbi.nih.gov/Bacteria/. Accession numbers are in parenthesis: *Desulfotomaculum acetoxidans* (NC_013216); *Desulfotomaculum kuznetsovii* (NC_015573); *"Desulfotomaculum reducens"* (NC_009253); *Pelotomaculum thermopropionicum* (NC_009454).
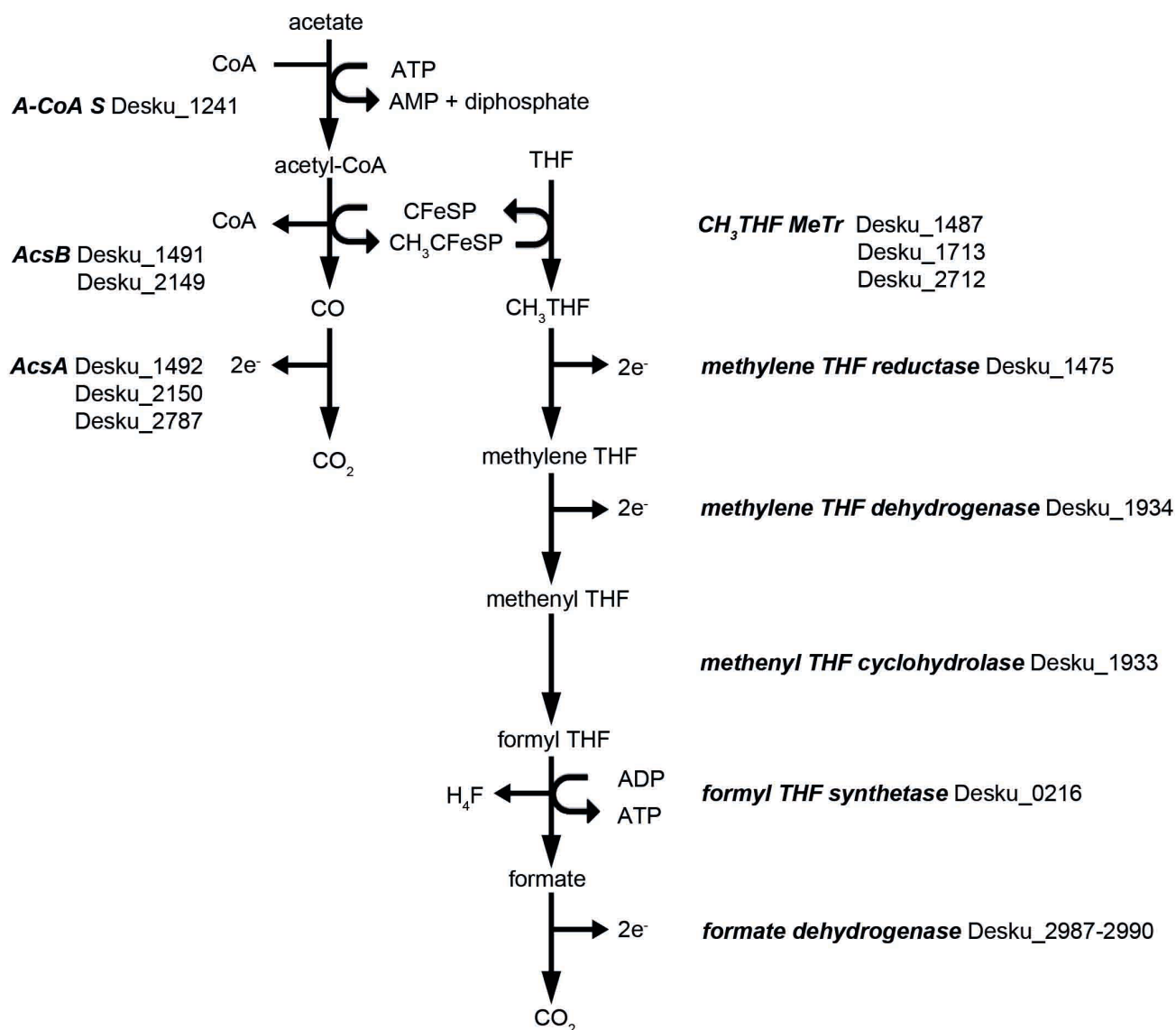
Figure 4. Pathway of acetate oxidation to CO_2 by *D. kuznetsovii*. Enzymes in this figure are in bold italic and their locus tags are included. Genes with the locus tags Desku_1488 and Desku_1490 putatively code for the small subunit and the large subunit of the iron-sulfur protein, respectively. This protein is involved in transferring the methyl from acetyl-CoA to tetrahydrofolate. Abbreviations: A-CoA S, acetyl-CoA synthetase; AcsA, carbon-monoxide dehydrogenase; AcsB, acetyl-CoA synthase; CFeSP, iron-sulfur protein; CH_3, methyl; THF, tetrahydrofolate; MeTr, methyltransferase.

Methanol metabolism

Growth of *D. kuznetsovii* with methanol and sulfate was studied [35]. In that study the activity of methyltransferase, an enzyme that is involved in methanol metabolism in methanogens and acetogens [36,37], could not be assessed, while low activities of an alcohol dehydrogenase could be measured. An alcohol dehydrogenase with a molecular mass of 42 kDa was partially purified and showed activity with methanol [35]. The genome of *D. kuznetsovii* contains several alcohol dehydrogenase genes (Desku_0165, 0619, 0624, 0628, 2955, 3082) that each code for an enzyme with a size of approximately 42 kDa. In the genome, genes with similarity to those coding for a methanol methyltransferase mtaA (Desku_0050, 0055, 0060), mtaB (Desku_0051) and mtaC (Desku_0048, 0049, 0052, 0056) were also found, suggesting a methanol metabolism as described in *Moorella thermoacetica* [36]. Further studies are needed to obtain information about the diversity of the methanol-degradation pathways in *D. kuznetsovii*.

Comparison of *D. kuznetsovii* and *P. thermopropionicum* genomes

Genomic comparison revealed that a large number of *D. kuznetsovii* genes show similarity to genes of *Pelotomaculum thermopropionicum*, a syntrophic propionate-oxidizing thermophile (Table 5 and 6). Interestingly, among them are genes that putatively code for enzymes involved in propionate metabolism (Table 7). Moreover, the genetic organization of the methylmalonyl-CoA (*mmc*) cluster in the genome of both bacteria is similar (Figure 5). However, *D. kuznetsovii* lacks *tps*, *mmcA* and *mmcM* in the *mmc* cluster. *mmcA* codes for a response regulator and *mmcM* for pyruvate ferredoxin oxidoreductase.

Based on 16S rRNA gene sequences, *D. kuznetsovii* and *P. thermopropionicum* group in cluster group c and h of the *Desulfotomaculum* cluster 1, respectively (see Figure 1). *P. thermopropionicum* is known for its ability to grow with propionate and ethanol in syntrophic association with methanogens. It is not able to grow by sulfate respiration, despite the presence of sulfate reduction genes in the genome [38]. In contrast, *D. kuznetsovii* is able to grow with propionate (Figure 6) and ethanol with sulfate. However, in the absence of sulfate, it cannot grow in syntrophic association with methanogens. Therefore, differences are expected in genes coding for hydrogenases, formate dehydrogenases, and those involved in sulfate reduction.

Sulfate reduction genes: Figure 7 depicts the sulfate reduction pathway of the two strains. In the genome of *D. kuznetsovii* two genes (Desku_2103; Desku_3527) are annotated as phosphoadenosine phosphosulfate reductase encoding genes whose corresponding proteins might be involved in assimilatory sulfate metabolism. The *P. thermopropionicum* genome lacks these genes [39]. Instead, the *P. thermopropionicum* genome contains an adenylylsulfate kinase gene (PTH_0238). In the dissimilatory sulfate reduction pathway, the two strains both have genes that code for enzymes to reduce sulfate to H_2S. However, *P. thermopropionicum* is missing the gene that codes for an adenylylsulfate reductase beta subunit, which is present in the *D. kuznetsovii* genome (Desku_1073). Moreover, the gene labeled as a dissimilatory sulfite reductase (dsr) alpha and beta subunit in the *P. thermopropionicum* genome (PTH_0242) is not similar to *dsrA or dsrB* from *D. kuznetsovii* or any other *Desulfotomaculum* strain.

However, it has high similarity to the *dsrC* gene from *D. kuznetsovii*, indicating that it is not a *dsrA* or *dsrB* gene but a *dsrC* gene (data not shown). Therefore, the inability of *P. thermopropionicum* to grow by sulfate respiration is most likely caused by the absence of an adenylylsulfate reductase beta subunit encoding gene and the *dsrAB* genes.

Hydrogenase and formate dehydrogenase genes: Schut and Adams (2009) [40] showed that the trimeric [FeFe]-hydrogenase from *Thermotoga maritima* oxidizes NADH and ferredoxin simultaneously to produce H_2. Similar bifurcating / confurcating [FeFe]-hydrogenases and formate dehydrogenases are present in *Syntrophobacter fumaroxidans* and *P. thermopropionicum* [41]. Both generate NADH and ferredoxin during propionate degradation via the methylmalonyl-CoA pathway and might use confurcating hydrogenases and formate dehydrogenases to drive the unfavorable re-oxidation of NADH (E_0'=-320mV) by the exergonic re-oxidation of ferredoxin (E_0'=-398mV) to produce hydrogen (E_0'= -414mV) or formate (E_0'= -432mV) that are subsequently transferred to hydrogen and formate scavenging methanogens. Additionally, up-regulation of genes encoding hydrogenases and formate dehydrogenases in *P. thermopropionicum* was shown during syntrophic growth [42]. The *P. thermopropionicum* genome contains three [FeFe]-hydrogenases, one [NiFe]-hydrogenase and two formate dehydrogenases. One [FeFe]-hydrogenase (PTH_0668-0670) was shown to be down-regulated during syntrophic growth, while the other two [FeFe]-hydrogenases (PTH_1377-1379 and PTH_2010-2012) were up-regulated. The two formate dehydrogenases of *P. thermopropionicum* (I, PTH_1711-1714 and II, PTH_2645-2649) were both up-regulated during syntrophic growth [42]. According to TMHMM server v. 2.0 [43] formate dehydrogenase I of *P. thermopropionicum* has transmembrane helices. Therefore, it might play an essential role in the interspecies transfer of reducing equivalents in syntrophic growth.

The genome of *D. kuznetsovii* was screened for hydrogenase and formate dehydrogenase encoding gene clusters with BLAST analysis. Pfam search [44] was used to identify motifs in the amino acid sequences and the TMHMM Server v. 2.0 [43] was used to screen for transmembrane helices. The TatP 1.0 Server was used to screen for twin-arginine translocation (Tat) motifs in the N-

terminus to predict protein localization in the cell [45]. The incorporation of selenocysteine (SeCys) was examined by RNA loop predictions with Mfold version 3.2 [46, 47]. The predicted RNA loop in the 50-100 bp region downstream of the UGA-codon was compared with the consensus loop described earlier [48].
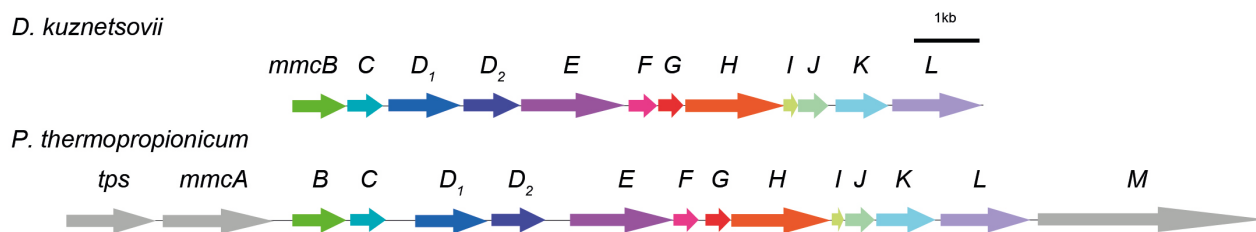

Figure 5. Gene organization of the *mmc* cluster in *D. kuznetsovii* and *P. thermopropionicum*. Names of the genes can be found in table 6, except for *tps*, which is a transposase gene.
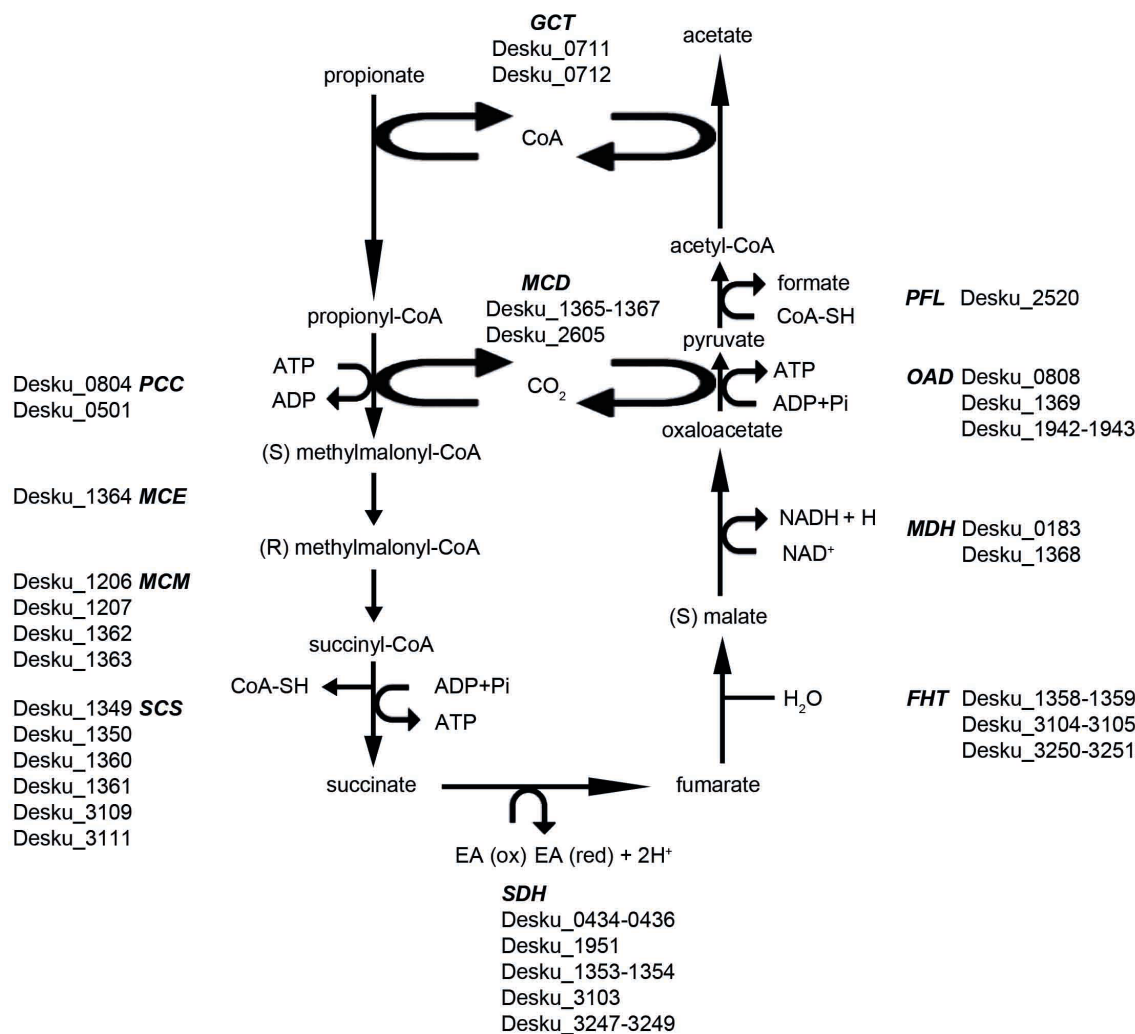
Figure 6. Propionate degradation pathway in *D. kuznetsovii* based on genomic data. Enzymes are depicted in bold italic. Next to these enzymes are the possible encoding genes, and their locus tags. GCT, Glutaconate CoA-transferase; MCD, Methylmalonyl-CoA decarboxylase; PCC, Propionyl-CoA carboxylase; MCE, Methylmalonyl-CoA epimerase; MCM, Methylmalonyl-CoA mutase; SCS, Succinyl-CoA synthetase; SDH, Succinate dehydrogenase; FHT, Fumarase; MDH, Malate dehydrogenase; OAD, Oxaloacetate decarboxylase; PFL, Pyruvate formate lyase.

Table 7. Genes in *D. kuznetsovii* that are annotated as enzymes involved in propionate metabolism[†].

| Gene symbol	Locus tag	Function	Homologous protein in *P. thermopropionicum*	
			Identity (%)	Locus tag
sdhB	Desku_0434	Succinate dehydrogenase, FeS protein	76	PTH_1018
sdhA	Desku_0435	Succinate dehydrogenase, flavoprotein	76	PTH_1017
sdhC	Desku_0436	Succinate dehydrogenase, cytochrome b	51	PTH_1016
citE	Desku_1348	Citrate lyase	57	PTH_1335
sdhA	Desku_1353	Succinate dehydrogenase, flavoprotein	83	PTH_1491
sdhB	Desku_1354	Succinate dehydrogenase, FeS protein	75	PTH_1490
mmcB	Desku_1358	Fumarase, N-terminal domain	73	PTH_1356
mmcC	Desku_1359	Fumarase, C-terminal domain	77	PTH_1357
mmcD2	Desku_1361	Succinyl-CoA synthetase, alpha subunit	78	PTH_1359
mmcE	Desku_1362	Methylmalonyl-CoA mutase, N-terminal domain	77	PTH_1361
mmcF	Desku_1363	Methylmalonyl-CoA mutase, C-terminal domain	82	PTH_1362
mmcG	Desku_1364	Methylmalonyl-CoA epimerase	86	PTH_1363
mmcH	Desku_1365	Methylmalonyl-CoA decarboxylase, alpha subunit	75	PTH_1364
mmcI	Desku_1366	Methylmalonyl-CoA decarboxylase, epsilon subunit	82	PTH_1365
mmcJ	Desku_1367	Methylmalonyl-CoA decarboxylase, gamma subunit	56	PTH_1366
mmcK	Desku_1368	Malate dehydrogenase	75	PTH_1367
mmcL	Desku_1369	Transcarboxylase 5S subunit	66	PTH_1368
pykF	Desku_1651	Pyruvate kinase	73	PTH_2214
ppsA	Desku_2615	Pyruvate phosphate dikinase	78	PTH_0903
citE	Desku_2747	Citrate lyase	56	PTH_1335

[†]Corresponding homologs in *P. thermopropionicum* are included.
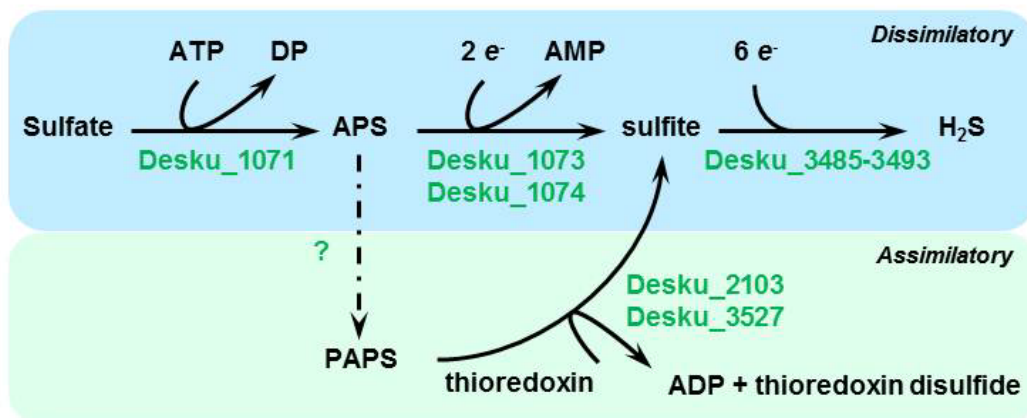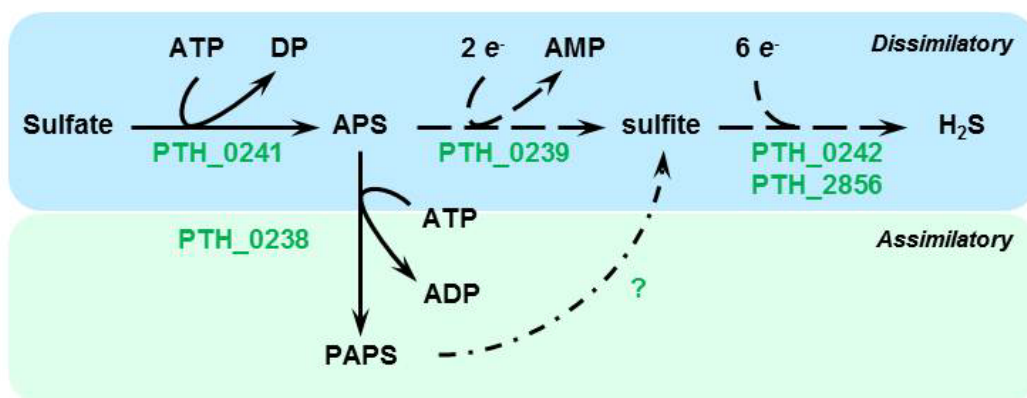
Figure 7. Sulfate reduction pathway of *D. kuznetsovii* and *P. thermopropionicum*. Depicted in green are genes that code for sulfate reduction enzymes that are present in the genome. Dashed arrows indicate the presence of a subunit encoding gene, but not the presence of all genes required for the enzyme. Dashed dotted arrows are used when no genes were found for the reaction. Abbreviations: APS, adenylylsulfate; DP, diphosphate; PAPS, 3'-Phosphoadenylyl-sulfate (PAPS); redA, reduced acceptor; oxA, oxidized acceptor.

Compared to *P. thermopropionicum*, *D. kuznetsovii* lacks membrane associated formate dehydrogenases and hydrogenases and also lacks [NiFe]-hydrogenase. This might explain why *D. kuznetsovii* cannot grow in syntrophic relation with methanogens. The genome of *D. kuznetsovii* indicates the presence of a confurcating selenocysteine-incorporated formate dehydrogenase (Desku_2987-2991), two trimeric confurcating [FeFe]-hydrogenases (Desku_2307-2309, Desku_2995-2997) and two [FeFe]-hydrogenases (Desku_0995, Desku_2934-2935) without NADH-binding sites (Figure 8). Several subunits of these enzymes are related to subunits of NADH dehydrogenase (complex I), including the NADH-binding proteins related to NuoF (Desku_2990, 2308 and 2996) and the electron transfer subunits related to NuoE (Desku_2991, 2935, and 2997) and to NuoG (Desku_2989). In three of the [FeFe]-hydrogenases this NuoG-like domain is fused with the catalytic subunit (Desku_2995, 2307 and 2934). Two of the multimeric hydrogenases are found next to [FeFe]-hydrogenases containing PAS-sensor domains (Desku_2932 and Desku_2994), suggesting they are involved in the regulation of the synthesis of those hydrogenases. All complexes are predicted to be cytoplasmic and not membrane bound.
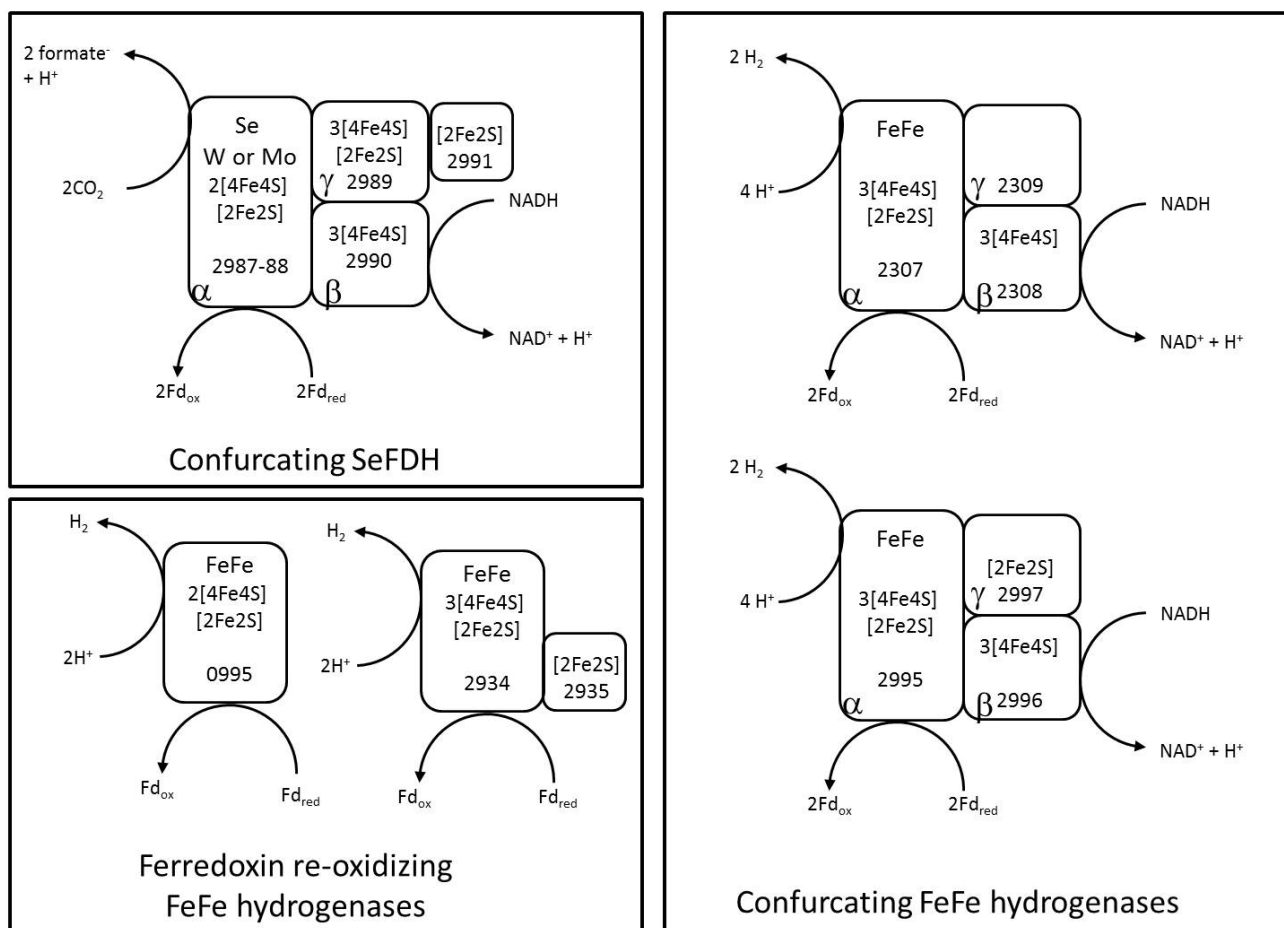
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Figure 8. Schematic representation of a putative confurcating formate dehydrogenase, two putative confurcating [FeFe]-hydrogenases and two ferredoxin re-oxidizing [FeFe]-hydrogenases in *Desulfotomaculum kuznetsovii*. Gene locus tag numbers and α-, β-, and γ-subunits are depicted. Moreover, predicted iron-sulfur clusters and metal-binding sites are indicated.

Apart from a possible involvement in the acetate oxidation pathway (Figure 4), it remains unclear for which purpose *D. kuznetsovii* uses its confurcating formate dehydrogenase and hydrogenases because our genome analysis indicates that pyruvate oxidation during propionate degradation generates formate instead of ferredoxin (Figure 6).

Vitamin synthesis

D. kuznetsovii is able to grow in medium without vitamins [1]. This indicates that *D. kuznetsovii* is able to synthesize all the vitamins that are required for its metabolism and that vitamin synthesis genes should be present in the genome. Vitamin B_{12} is essential for the methylmalonyl-CoA

pathway and the acetyl-coA pathway. The biosynthesis of cobalamin (vitamin B_{12}) is known to occur from uroporphyrinogen-III to adenosylcobalamin via two possible pathways, the aerobic and anaerobic pathway of the corrinoid ring [49,50]. The *D. kuznetsovii* genome contains all genes needed for the anaerobic pathway: *cysG^A* (Desku_1520), *cysG^B* (Desku_1460, Desku_1523), *cbiA* (Desku_1765, Desku_2368), *cbiBCDEFGHJLPT* (Desku_2369, 1459, 1468, 1467, 1464, 1463, 1462, 1461, 1465, 2370 and 1466, respectively), cobalt reductase (Desku_2757), *btuR* (Desku_0004, 1209), *cobS* (Desku_2367) and *cobU* (Desku_2371). Moreover, *D. kuznetsovii* has genes to convert glutamyl tRNA to uroporphyrinogen-III, *hemABCDL* (Desku_1522, 1518, 1521, 1520 and 1522, respectively). The genome also contains

some unassigned cobalamin synthesis genes (P47K, Desku_0046, 0053; cbiM, Desku_2905), corrinoid transport proteins (Desku_0693, 702, 2237-2239, 2902-2904, 3025-3027) and, interestingly, two cobN genes (Desku_2189, 2227), genes involved in the aerobic pathway. It is unclear why *D. kuznetsovii* has these cobN genes, since all anaerobic pathway genes are present in the genome, and it is unclear if the products of these two genes are used for cobalamin synthesis by *D. kuznetsovii*.

Other vitamin synthesis genes present in the genome of *D. kuznetsovii* are genes involved in biotin synthesis (vitamin H) (Desku_1295-1297, 2246-2247, 2317), nicotinamide (vitamin B_3) synthesis (Desku_0433, 0614, 0662, 0815, 1248, 1417, 1472, 1499, 1925, 1951, 3103, 3121, 3227, 3228, 3231, 3246, 3337), thiamin (vitamin B_1) synthesis (0372, 0543, 0545, 2253, 2363, 2639), riboflavin (vitamin B_2) synthesis (Desku_1244-1247), and pantothenate (vitamin B_5) synthesis (Desku_3262). The genes involved in coenzyme A production from pantothenate are also present in the *D. kuznetsovii* genome (Desku_1254, 1307, 3145, 3200). Moreover, genes involved in the biosynthesis of pyridoxine (vitamin B_6) via the deoxyxylulose 5-phosphate (DXP) independent route were found to be in the genome (Desku_0007, 0008). These genes code for two enzymes that facilitate the conversion of glutamine to the active form of vitamin B_6, pyridoxal 5'-phosphate [51].

Menaquinone (vitamin K) and ubiquinone (coenzyme Q_{10}) biosynthesis is important because of the electron transport function in the membranes. The genes that code for the biosynthesis enzymes from polyprenyldiphosphate to menaquinone and ubiquinone are present in the *D. kuznetsovii* genome (Desku_0124, 0126, 0629, 1551-1554, 1829, 2629 and 3525), except for the genes that code for a 2-polyprenyl-6-methoxyphenol 4-monooxygenase (UbiH) and 2-polyprenyl-3-methyl-6-methoxy-1,4-benzoquinone hydroxylase (UbiF). Additionally, three genes (Desku_1548-1550) could be identified as putative menaqui-

none biosynthesis genes and are part of a menaquinone biosynthesis gene cluster (Desku_1548-1554). The products of those three genes could be involved in the reactions of the missing UbiH and UbiF encoding genes.

Folate (vitamin B_9) biosynthesis is also of great importance for *D. kuznetsovii*, because it is an essential part of the acetyl-CoA pathway. It is involved in the transfer of one-carbon compounds and can be biosynthesized from chorismate and guanosine triphosphate (GTP) [52-55]. Both pathways use a dihydropteroate synthase to produce dihydropteroate. The genome of *D. kuznetsovii* contains the genes encoding the enzymes involved in the pathway from chorismate to dihydropteroate (Desku_0219, 2268-2269) and from GTP to dihydropteroate (Desku_0210, 0219-0221 and 1419). The gene encoding a phosphatase (Desku_0210) in the *D. kuznetsovii* genome is probably involved in the removal of phosphate groups from dihydropterine triphosphate as a substitute for an alkaline phosphatase encoding gene, which is not present in the genome. Additionally, the genome contains a bifunctional protein encoding gene (Desku_404) that is expected to be responsible for the production of dihydrofolate (DHF) and the addition of multiple glutamate moieties to DHF or tetrahydrofolate (THF). However, the *D. kuznetsovii* genome lacks the DHF reductase encoding gene, which is required to reduce DHF to THF. The DHF reductase encoding gene appears to be absent in many microorganisms [56]. Levin et al. (2004) propose that in *Halobacterium salinarum* a dihydrofolate synthase and a dihydropteroate synthase domain is able to replace the function of the DHF reductase. Additionally, the authors show that when using a BLAST search, homologs of polypeptides can be found in organisms that lack a DHF reductase [56]. However, BLAST results showed no homologous protein encoding gene in the genome of *D. kuznetsovii* (data not shown). How in *D. kuznetsovii* DHF is reduced to THF can currently not be deduced from the genome sequence.

Acknowledgements

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