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Global transcriptomics analysis of the *Desulfovibrio vulgaris* change from syntrophic growth with *Methanosarcina barkeri* to sulfidogenic metabolism

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Desulfovibrio vulgaris is a metabolically flexible micro-organism. It can use sulfate as an electron acceptor to catabolize a variety of substrates, or in the absence of sulfate can utilize organic acids and alcohols by forming a syntrophic association with a hydrogen-scavenging partner to relieve inhibition by hydrogen. These alternative metabolic types increase the chance of survival for *D. vulgaris* in environments where one of the potential external electron acceptors becomes depleted. In this work, whole-genome *D. vulgaris* microarrays were used to determine relative transcript levels as *D. vulgaris* shifted its metabolism from syntrophic in a lactate-oxidizing dual-culture with *Methanosarcina barkeri* to a sulfidogenic metabolism. Syntrophic dual-cultures were grown in two independent chemostats and perturbation was introduced after six volume changes with the addition of sulfate. The results showed that 132 genes were differentially expressed in *D. vulgaris* 2 h after addition of sulfate. Functional analyses suggested that genes involved in cell envelope and energy metabolism were the most regulated when comparing syntrophic and sulfidogenic metabolism. Upregulation was observed for genes encoding ATPase and the membrane-integrated energy-conserving hydrogenase (Ech) when cells shifted to a sulfidogenic metabolism. A five-gene cluster encoding several lipoproteins and membrane-bound proteins was downregulated when cells were shifted to a sulfidogenic metabolism. Interestingly, this gene cluster has orthologues found only in another syntrophic bacterium, *Syntrophobacter fumaroxidans*, and four recently sequenced *Desulfovibrio* strains. This study also identified several novel *c*-type cytochrome-encoding genes, which may be involved in the sulfidogenic metabolism.

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INTRODUCTION

Dissimilatory sulfate-reducing bacteria (SRB) are a diverse group of obligate anaerobic bacteria that are found ubiquitously in nature and play an important role in the global cycling of carbon and sulfur. SRB belonging to the genus *Desulfovibrio* have been shown to possess distinct metabolic capabilities and ecological characteristics (Voordouw, 1995). The SRB mainly use sulfate as the terminal electron acceptor during the oxidation of various electron donors (Widdel & Hansen, 1991; Muyzer & Stams, 2008). Some SRB can use nitrate as an electron acceptor, and their possible microaerophilic nature has also been discussed (Cypionka, 2000). Generally, sulfate reducers can be divided into two main groups: those that degrade organic compounds incompletely to acetate and those that degrade organic compounds completely to carbon dioxide.

In marine sediments, sulfidogenic bacteria were thought to use all the products of primary fermentations and oxidize them to CO_2 (Muyzer & Stams, 2008). Where sediments are high in organic matter, sulfate is depleted at shallow sediment depths and biogenic methane production results.

Abbreviations: qRT-PCR, quantitative RT-PCR; SRB, sulfate-reducing bacteria.

Two supplementary tables, listing responsive genes in *D. vulgaris* involved in energy metabolism and the cell envelope, are available with the online version of this paper.

The microarray data discussed in this paper are available from ArrayExpress via accession number E-MTAB-304.

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In the absence of sulfate, *Desulfovibrio vulgaris* and SRB in general ferment organic acids and alcohols, producing hydrogen, acetate and carbon dioxide, and rely on hydrogen- and acetate-scavenging methanogens to convert these compounds to methane (Bryant *et al.*, 1977; McInerney *et al.*, 1981). This symbiotic process is known as 'syntrophy' and is a widespread microbial interaction, especially in methanogenic environments (Bryant *et al.* 1967; Schink 1997; Stams & Plugge, 2009). We can thus distinguish two major lifestyles for some SRB, and these are sulfidogenic and syntrophic metabolism. The advantage of having different metabolic capabilities is that it raises the chances of survival of SRB in environments where electron acceptors become depleted. In these marine sediments, SRB and methanogens do not compete but rather complement each other in the degradation of organic matter. Even in sulfate-rich marine sediments, SRB and methanogens co-exist, presumably by competing for common substrates, such as H_2 (Oremland & Polcin, 1982; Winfrey & Ward, 1983; Kuivila *et al.*, 1990; Holmer & Kristensen, 1994). Recently, it was found that sulfate reducers are still very abundant in the methanogenic zones of Aarhus Bay (Leloup *et al.*, 2009).

In the past decades significant progress has been made through extensive studies of monoculture metabolism (i.e. sulfidogenic metabolism) in SRB particularly with the model species *D. vulgaris* (Peck, 1966; Odom & Peck, 1981; Aubert *et al.*, 2000; Heidelberg *et al.*, 2004). Recently, research efforts on SRB were greatly facilitated by the release of the *D. vulgaris* genome (Heidelberg *et al.*, 2004). Since then, several groups have reported global transcriptomic and proteomic analyses of *D. vulgaris* under various growth or stress conditions (Chhabra *et al.*, 2006; Clark *et al.*, 2006; He *et al.*, 2006; Mukhopadhyay *et al.*, 2006, 2007; Zhang *et al.*, 2006a, b, c; Bender *et al.*, 2007; Tang *et al.*, 2007; Pereira *et al.*, 2008; Walker *et al.*, 2009). As a result, there has been a better understanding of the electron transfer and energy conservation mechanisms of *D. vulgaris* associated with lactate oxidation during sulfidogenic growth. Yet, Pereira *et al.* (2008) highlighted that the energy metabolism of *D. vulgaris* is very complex and flexible, and as such deserves further study.

While the physiology of the symbiotic relationship has been studied for more than 40 years (Bryant *et al.*, 1967; Stams, 1994; Schink, 1997; Stams & Plugge, 2009), relatively little is known about the genes involved in syntrophic interactions (Schink, 2002), which may be due to lack of methodologies for large-scale measurement of biological properties in mixed-culture systems. The availability of complete genome sequences has enabled global gene expression studies and protein abundance analysis of mixed-culture systems. In a recent study, comparative transcriptional analysis of *D. vulgaris* in two culture conditions was performed: syntrophic dual-cultures with a hydrogenotrophic methanogen *Methanococcus maripaludis* strain S2 (lacking sulfate) and sulfate-limited monocultures (Walker *et al.*, 2009). The results showed that during syntrophic growth on lactate with a hydrogenotrophic methanogen, numerous genes involved in electron transfer and energy generation are upregulated in *D. vulgaris* compared with their expression in sulfate-limited monocultures. In addition, the results also demonstrated that syntrophic growth and sulfate respiration use largely independent energy-generation pathways, implying that the molecular mechanism of microbial syntrophic processes cannot be fully deciphered by studying pure cultures alone.

We have been working with *D. vulgaris* and its dual-culture with *Methanosarcina barkeri* in recent years (Culley *et al.*, 2006; Scholten *et al.*, 2007a, b; Zhang *et al.*, 2006a, b, d). To further explore the metabolic and regulatory mechanisms associated with the syntrophic metabolism, we performed a global transcriptomic analysis of *D. vulgaris* during its metabolic shift from syntrophic growth with *M. barkeri* to sulfidogenic growth. Instead of establishing two simultaneous cultures (i.e. syntrophic dual-culture versus *D. vulgaris* monoculture), we grew syntrophic dual-cultures in chemostats, and after six volume changes, sulfate was added to the chemostats. Thus, when *D. vulgaris* changed to the sulfidogenic metabolism it remained in the presence of *M. barkeri*, as occurs in natural ecosystems. The purposes of this investigation were to: (i) examine the energy-yielding metabolic pathways involved in syntrophic growth on lactate; and (ii) seek global information regarding the gene expression response of *D. vulgaris* during its change from syntrophic to sulfidogenic metabolism.

METHODS

Cultivation. *D. vulgaris* subsp. *vulgaris* strain Hildenborough (DSM 644) and *M. barkeri* strain Fusaro (DSM 804) were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and grown in bicarbonate-buffered sulfide-reduced mineral medium as described previously by Scholten & Conrad (2000). Pure cultures of *D. vulgaris* and *M. barkeri* were maintained routinely on lactate (40 mM) plus sulfate (20 mM) and H_2 plus CO_2 (80 : 20), respectively. Continuous cultivation experiments were performed in duplicate with dual-cultures of *D. vulgaris* and *M. barkeri* in 7.5 l Bioflow fermenters (New Brunswick) with a working volume of 4000 ml under similar conditions to those described by Scholten & Conrad (2000). The dilution rate of the chemostats was set at 0.4 d^{-1}. In order to study the metabolism change of *D. vulgaris* from syntroph to sulfidogen, dual-cultures were initially grown under lactate limitation and absence of sulfate, i.e. syntrophic conditions (days 0–31). Then the syntrophic cultures were perturbed by adding sulfate (15 mM final concentration) to the chemostats and supply medium (day 31). The dual-cultures were further grown under lactate limitation but with excess of sulfate, i.e. sulfidogenic conditions (days 31–67). Cells from different growth conditions were harvested from the chemostats for subsequent microarray analysis: syntrophic phase (t=17 days), perturbation phase (2 h after perturbation at t=31 days) and sulfidogenic phase (t=66 days). Steady-state conditions for syntrophic phase were maintained for at least six volume changes. Substrate consumption and product formation were monitored, and total cell mass and species composition were checked under all steady-state conditions

according to methods described previously (Scholten & Conrad, 2000). The experiments were performed at 35 °C. Cells were transferred to centrifuge bottles with O-ring seals in an anaerobic hood and collected by centrifuging at room temperature (6000 ***g***). The supernatant was removed in the anaerobic hood and the cell pellet was immediately frozen at −80 °C. Each sample used for RNA profiling was a biological replicate. RNA isolation was performed as described previously (Zhang *et al.*, 2006a; b; Culley *et al.*, 2006). To further assess if the genes preferentially expressed during syntrophic growth were associated exclusively with syntrophy, the transcriptional response of these genes was also checked under a number of *D. vulgaris* monoculture growth conditions (Zhang *et al.*, 2006a, b).

Generation of the *D. vulgaris* microarray. Microarrays were designed by NimbleGen System using their Maskless Array Synthesizer (MAS) technology (Nuwaysir *et al.*, 2002; Zhang *et al.*, 2006a; Scholten *et al.*, 2007b). The *D. vulgaris* genome sequence was obtained from the Institute for Genomics Research (TIGR) (Heidelberg *et al.*, 2004). The array containing 3548 ORFs was manufactured as described by Nuwaysir *et al.* (2002). Arrays were designed with JazzSuite software and the MAS units were used to manufacture the custom arrays. For each ORF, 18 unique 24-mer oligonucleotides from throughout the ORF were printed onto glass microscope slides.

RNA isolation. Frozen dual-culture cell pellets (250–500 µl) were ground to a fine powder with liquid nitrogen in a mortar and pestle. A 1 ml volume of TRIzol reagent (Invitrogen) was immediately added to the powder in the pestle and allowed to thaw. The resulting slurry was transferred to a 2 ml O-ring tube containing 100 µl each of 0.5 and 0.1 mm glass/zirconia beads and homogenized for 6 min (twice for 3 min with 5 min rest between) in a Mini-BeadBeater 8 cell disruptor (Biospec Products) at maximum speed. The tubes were then incubated at room temperature for 5 min before addition of 200 µl chloroform, vortexing for 15 s and centrifugation at 12 000 ***g*** for 15 min at room temperature. The aqueous layer (~600 µl) was transferred to a tube containing 600 µl 2-propanol, mixed and incubated at room temperature for 15 min before centrifugation at 12 000 ***g*** for 15 min at 4 °C. The pellet was washed with 70 % ethanol, air-dried and resuspended in 50–100 µl RNase-free water by heating to 60 °C for 10 min. The concentration and purity of the RNA were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies) and the integrity was verified by gel electrophoresis. The quality of total RNA obtained was checked using the Agilent 2100 Bioanalyser (Agilent Technologies) before it was used for RNA labelling (Culley *et al.*, 2006).

RNA biotin labelling. The total RNA of dual-culture cells was biotin-labelled via first-strand cDNA synthesis, cDNA digestion, and a terminal transferase end-label reaction with biotinylated ddATP (Nuwaysir *et al.*, 2002; Albert *et al.*, 2003; Zhang *et al.*, 2006a). The amounts of RNA used for labelling and hybridization were normalized by the cell ratio between *D. vulgaris* and *M. barkeri* so that they could be compared consistently across different samples.

Hybridization and washing. Microarrays were hybridized with 10–20 µg biotinylated cDNA in 300 µl, in the presence of 50 mM MES, 0.5 M NaCl, 10 mM EDTA and 0.005 % (v/v) Tween-20 for 16 h at 45 °C. Before application to the array, samples were heated to 95 °C for 5 min, allowed to cool, heated to 45 °C for 5 min, and spun at 14 000 ***g*** for 5 min. Hybridization was performed with agitation in disposable adhesive hybridization chambers from Grace BioLabs in a hybridization oven. After hybridization, arrays were washed in non-stringent (NS) buffer [6 × sodium chloride, sodium phosphate and EDTA (SSPE), 0.01 % (v/v) Tween-20] for 5 min at room temperature, followed by washing in stringent buffer (100 mM MES, 0.1 M NaCl, 0.01 % Tween-20) for 30 min at 45 °C. After washing, arrays were stained with streptavidin–cy3 conjugate from Amersham Pharmacia for 25 min at room temperature, followed by a 5 min wash in NS buffer, a 30 s rinse in 1 × NimbleGen final rinse buffer, and a blow-dry step using high-pressure grade-5 Argon (Badger Welding) (Zhang *et al.*, 2006a; Scholten *et al.*, 2007b).

Microarray data normalization and gene expression analysis. Prior to data extraction, images were rotated and doubled in size (without interpolation) using ImageJ software (http://rsb.info.nih.gov/ij/). Features were extracted using GenePix 3.0 software (AxonInstruments) using a fixed feature size. The local background correction from the GenePix software was not applied to raw signal intensities. The data were normalized using tools available through the Bioconductor project (http://www.bioconductor.org), as described elsewhere (Nuwaysir *et al.*, 2002; Albert *et al.*, 2003). The gene calls were based on the Bioconductor implementation of the MAS 5 algorithms. For each experimental condition two biological replicates were collected and used for RNA isolation. In addition, each biological replicate was analysed twice on the microarray. In total, four microarray measurements were obtained for each sampling point. Three pair-wise comparisons were performed: (i) perturbation versus syntrophic; (ii) sulfidogenic versus syntrophic; and (iii) perturbation versus sulfidogenic. For each pair-wise comparison, raw intensity microarray data were normalized by taking a $\log_2$ transformation and used in a two-sample *t* test for each gene. A *P* value was reported for each gene (Simon *et al.*, 2003). *P* values for all genes across the genome were adjusted to account for multiple testing (Benjemini & Hochberg, 1995). Genes were classified as differentially expressed based on a *P*-value criterion of less than 0.1. In addition, the fold change of expression of any given gene was calculated using raw intensity data. All computations were performed in SAS (SAS Institute) and the program is available upon request from the authors.

Real-time PCR analysis. Verification of the microarray results for a selection of *D. vulgaris* genes was initially performed using TaqMan quantitative RT-PCR (qRT-PCR), as described previously (Scholten *et al.*, 2007a). Briefly, RNA from each time point used in the microarray analysis was converted to cDNA using random primers and the StrataScript qPCR cDNA Synthesis kit (Stratagene) according to the manufacturer's instructions. The reactions were carried out in an ABI 7700 Sequence Detector (Perkin-Elmer/Applied Biosystems) using the Brilliant qPCR Master Mix kit from Stratagene according to the manufacturer's instructions. The reaction conditions used were as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 25 s and 60 °C for 1.5 min. The resulting threshold cycle (Ct) data were normalized relative to 16S rRNA levels using the ΔCt method as described in the Applied Biosystems User Bulletin #3 (Perkin-Elmer/Applied Biosystems). The ΔCt method was used for relative quantification of transcripts (Livak & Schmittgen, 2001). In addition, SYBR-based qRT-PCR was also performed for the selected genes on an ABI StepOne Real-Time PCR system (Applied Biosystems). The iQ SYBR Green Supermix and iScript SYBR Green RT-PCR kit were purchased from Bio-Rad. The genes selected for qRT-PCR analysis are discussed in the text and listed in Table 3.

RESULTS AND DISCUSSION

Chemostat performance

Here we performed experiments with *D. vulgaris–M. barkeri* dual-culture and determined *D. vulgaris* gene expression patterns during its change from syntrophic to sulfidogenic metabolism using DNA microarrays and real-time RT-PCR. To obtain biomass for transcriptomic

Table 1. Numbers of genes differentially expressed in *D. vulgaris* under various conditions

Cellular role	Number of genes differentially expressed			
	Perturbation vs syntrophic	Sulfidogenic vs syntrophic	Perturbation vs sulfidogenic	Total
Amino acid biosynthesis	1	29	12	79
Biosynthesis of cofactors, prosthetic groups and carriers	2	34	19	97
Cell envelope	11	109	44	263
Cellular processes	6	48	20	158
Central intermediary metabolism	2	11	12	40
DNA metabolism	3	22	5	88
Energy metabolism	20	87	30	256
Fatty acid and phospholipid metabolism	0	6	4	24
Hypothetical proteins	32	436	157	1228
Other categories	7	40	5	105
Protein fate	5	44	17	124
Protein synthesis	15	44	16	133
Purines, pyrimidines, nucleosides and nucleotides	1	13	5	42
Regulatory functions	5	53	17	165
Signal transduction	0	14	4	52
Transcription	2	7	7	31
Transport and binding proteins	5	71	31	240
Unknown function	15	134	59	382
Total	132	1202	464	3507

analysis, chemostat cultures of syntrophically growing *D. vulgaris* and *M. barkeri* were produced. Four days after the establishment of the dual-culture chemostat, steady-state concentrations of lactate were below detectable levels, and acetate and CH_4 were formed (Fig. 1). The biomass ratio between *D. vulgaris* and *M. barkeri* was around 1 : 1. During the whole chemostat run, conversion of substrates to products was generally well balanced (C balance 90–110 % and electron balance 96–105 %). The *D. vulgaris* lifestyle change from syntrophic to sulfidogenic at $t=31$ days was confirmed by analysing the chemostat performance (Fig. 1). Directly after the addition of sulfate (day 31) to the chemostat and supply medium, *D. vulgaris* began producing sulfide. Two days after sulfate addition, the active biomass in the chemostat converted lactate plus sulfate to acetate and sulfide (Fig. 1). After perturbation, a significant decrease of *M. barkeri* biomass was observed by microscopy, presumably due to inactive or dying *M. barkeri* cells washing out of the chemostats, which is consistent with upregulation of *M. barkeri* genes involved in cell ageing and death at $t=66$ days, such as genes encoding products involved in protein degradation and recycling (*M. barkeri* microarray data not shown, available upon request).

General patterns of gene expression

Three pair-wise comparisons of microarray data were performed and the genes differentially expressed are presented by functional category in Table 1. Using a $P<0.1$ cut-off, a total of 132, 1202 and 484 genes (4–34 % of the genome) were identified as responsive in the pair-wise comparison of the perturbation versus syntrophic, sulfidogenic versus syntrophic, and perturbation versus sulfidogenic phases, respectively (Table 1). For most of the differentially expressed genes, the changes were relatively moderate, with fold-changes ranging from 1.1 to 3.1. Responsive genes were found in almost all aspects of *D. vulgaris* metabolism. Based on the percentage of the responsive genes in each functional category, the most broadly affected categories were hypothetical proteins, proteins with unknown functions, and cell envelope and energy metabolism proteins (Table 1). It is not very common that the top categories affected by the growth condition change are hypothetical proteins and proteins of unknown functions; however, this may suggest that the metabolic mechanisms of syntrophic metabolism in *D. vulgaris* are different from those known for sulfidogenic metabolism in monoculture.

Energy metabolism is responsive to the lifestyle change

Twenty-one genes involved in energy metabolism were upregulated after perturbation of the syntrophic dual-cultures with sulfate, and remained upregulated in the sulfidogenic phase, indicating their possible role in sulfidogenic growth of *D. vulgaris* on lactate (Table 2). These included three genes encoding various components of an Ech hydrogenase, four genes encoding various components of an F_0 or F_1 ATP synthase, three genes encoding proteins in the cytochrome *c* network, three genes encoding an unspecified reductase, one gene for a thiosulfate reductase, one gene for an iron–sulfur cluster binding protein, one gene for a

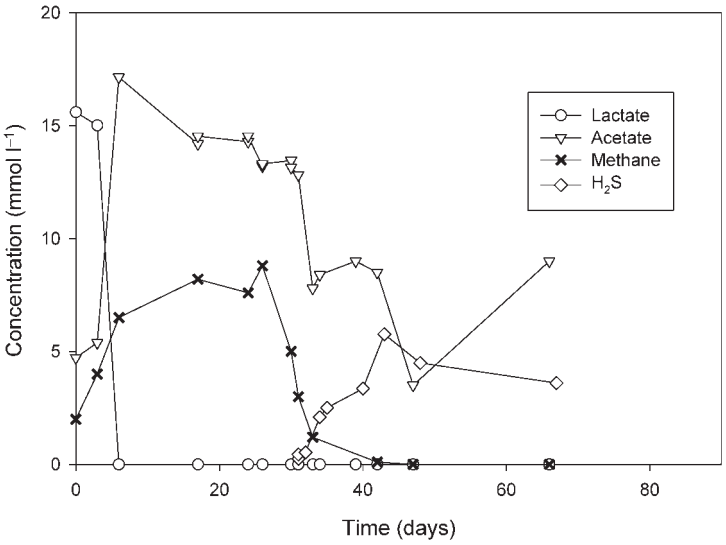

Fig. 1. Production of metabolites during growth of a *D. vulgaris* and *M. barkeri* co-culture: syntrophic (days 0–31) and sulfidogenic phase (days 31–67). Sulfate was added on day 31 to the chemostats and supply medium. The concentrations on the *y* axis are per litre medium.

ferredoxin, one gene for a nitrate reductase, and one gene of a fructose-1,6-bisphosphate aldolase. One gene involved in energy metabolism encoding high-molecular-mass (*hmc*) cytochrome *c* operon protein 4 was downregulated directly after perturbing the syntrophic dual-cultures with sulfate and remained downregulated in the sulfidogenic phase, indicating its role in syntrophic growth of *D. vulgaris* on lactate (Table 2). Several other genes involved in energy metabolism were up- or downregulated after the perturbation with sulfate, but these responsive genes were only observed in either the perturbation or sulfidogenic phase (Supplementary Table S1).

We expected that ATP synthesis would only take place via substrate-level phosphorylation during the syntrophic metabolism in *D. vulgaris*, while the sulfidogenic metabolism would require additional ATP generation from a proton motive force. Significant changes in gene expression levels were observed for four genes involved in ATP generation (Table 2). Two of these genes (DVU0777 and DVU0778) encode the F_1 alpha and delta subunit of the F_1 ATP synthase, and another two genes (DVU0779 and DVU0780) encode the F_0 beta subunit of the F_0 ATP synthase, respectively. These genes were expressed at 1.2–1.7-fold higher levels in the sulfidogenic metabolism relative to the syntrophic metabolism.

Interestingly, our microarray data showed considerable changes in gene expression levels for three genes encoding components of a membrane-bound hydrogenase (Ech). Upregulation of these genes suggests that the expression of Ech hydrogenase is associated with the *D. vulgaris* sulfidogenic metabolism (Table 2). The Ech hydrogenase is assumed to generate H_2 in the cytoplasm that is then captured by periplasmic hydrogenases to form a proton gradient (Heidelberg *et al.*, 2004). Our results support the hypothesis that additional ATP is only generated by proton gradient force in the *D. vulgaris* sulfidogenic metabolism. Upregulation of the Ech hydrogenase (DVU0431) and ATP synthase (DVU0777 and DVU0778) genes was also confirmed by qRT-PCR (Table 3). Several members of the Ech family of hydrogenases have been proposed to function in energy-conserving processes (Vignais *et al.*, 2001), and the Ech from *M. barkeri* has been shown to play a central and diverse role in its metabolism, including hydrogen formation from reduced ferredoxin with energy conservation, as well as reduction of ferredoxin by hydrogen via reverse electron transport (Meuer *et al.*, 1999, 2002). The *D. vulgaris ech* operon has the same organization as the homologous operon in *M. barkeri*, suggesting that they encode very similar hydrogenases that possibly play similar roles. Due to the sequence similarities to Complex I, it is also possible that Ech is functioning as a proton pump in *D. vulgaris*. Ech in *D. vulgaris* is expressed at lower levels during syntrophic growth, and is upregulated during perturbation and during the sulfidogenic metabolism. This suggests that the Ech hydrogenase from *D. vulgaris* may have a less prominent role in its metabolism than its counterpart in *M. barkeri*, although more evidence is still needed. Phylogenetic analysis based on the EchC amino acid sequence revealed that the *Desulfovibrio* EchC subunit was closely related to EchC of *M. barkeri* (Rodrigues *et al.*, 2003). This might be due to lateral gene transfer of the Ech-type hydrogenases during the evolutionary process, as already suggested by Vignais *et al.* (2001).

In a monoculture of *D. vulgaris*, the electrons generated during electron donor oxidation are channelled to sulfate through a vast network of haems that is created by various interconnected c_3-type cytochromes and involves several transmembrane complexes (Aubert *et al.*, 2000; Heidelberg *et al.*, 2004). As expected, our study showed that many genes from the *c*-type cytochrome network were upregulated in *D. vulgaris* during the perturbation and in the sulfidogenic metabolism, which suggests that an important part of the *c*-type cytochrome network may not be involved

Table 2. Key responsive genes in *D. vulgaris* following lifestyle changes

–, No change.

Gene ID	Description	Gene response		
		Perturbation vs syntrophic	Sulfidogenic vs syntrophic	Perturbation vs sulfidogenic
Energy metabolism				
DVU0173	Thiosulfate reductase, putative	1.18	1.10	–
DVU0259	DNA binding response regulator	1.83	1.30	1.41
DVU0260	Response regulator	1.50	–	1.45
DVU0261	Universal stress protein family	2.25	1.17	1.92
DVU0262	Hypothetical protein	1.46	1.35	–
DVU0263	Acidic cytochrome c_3	1.50	1.50	–
DVU0264	Ferredoxin, 4Fe–4S, putative	1.67	1.43	–
DVU0265	Membrane protein, putative	1.45	–	1.23
DVU0266	Hypothetical protein	1.54	1.19	1.30
DVU0267	Hypothetical protein	1.59	–	1.47
DVU0429	Ech hydrogenase, subunit EchF, putative	1.34	1.25	–
DVU0431	Ech hydrogenase, subunit EchD, putative	1.44	1.13	1.28
DVU0434	Ech hydrogenase, subunit EchA, putative	1.33	1.39	–
DVU0533	Hmc operon protein 4	−1.19	−1.27	–
DVU0777	ATP synthase, F_1 alpha subunit (*atpA*)	1.69	1.24	1.36
DVU0778	ATP synthase, F_1 delta subunit (*atpH*)	1.40	1.46	–
DVU0779	ATP synthase F_0, B subunit, putative	1.43	1.31	–
DVU0780	ATP synthase F_0, B subunit, putative	1.70	1.29	1.32
DVU1286	Reductase, transmembrane subunit, putative	1.31	1.00	1.31
DVU1287	Reductase, iron–sulfur binding subunit, putative	1.53	1.65	–
DVU1288	Cytochrome *c* family protein	1.40	1.36	–
DVU1289	Reductase, iron–sulfur binding subunit, putative	1.33	1.18	–
DVU1290	Nitrate reductase, gamma subunit, putative	1.32	1.15	–
DVU1770	Periplasmic [Fe] hydrogenase, small subunit (*hydB*)	–	1.21	–
DVU1782	Iron–sulfur cluster binding protein	1.13	1.17	–
DVU2143	Fructose-1,6-bisphosphate aldolase, class II (*fba*)	1.18	1.12	–
Cell envelope				
DVU0148	Lipoprotein, putative	−2.75	−3.10	–
DVU0149	Membrane protein, putative	−1.96	−1.96	–
DVU0150	Membrane protein, putative	−1.40	−1.39	–
DVU0163	Lipoprotein, putative	−1.19	−1.24	–
DVU0290	Lipoprotein, putative	1.20	1.37	–
DVU0761	Lipoprotein, putative	1.36	1.31	–
DVU2301	Lipoprotein, putative	−1.10	−1.15	–
DVU2506	UDP-*N*-acetylmuramoylalanine-D-glutamate ligase (*murD*)	−1.12	−1.14	–
DVU2958	Membrane protein, putative	1.25	1.58	−1.26
Iron transport and binding proteins				
DVU2571	Ferrous iron transport protein B (*feoB*)	2.01	1.62	1.24
DVU2572	Ferrous iron transport protein A (*feoA*)	1.46	1.14	1.28

in the transfer of electrons during the *D. vulgaris* syntrophic metabolism. In particular, nine adjacent genes (DVU0259–267) were upregulated by the sulfate perturbation. Six of these genes, most notably the acidic c_3-type cytochrome (DVU0263) and a putative 4Fe–4S ferredoxin (DVU0264), were also upregulated in the sulfidogenic versus syntrophic metabolism (Table 2). Interestingly, the responsive tetrahaem c_3-type cytochrome (DVU0263) is located in the same operon as a gene encoding ferrodoxin, which implies that this cytochrome accepts the electrons arising from lactate oxidation through a ferrodoxin. Recently, the isolation and characterization of the respiratory membrane complex TMC (DVU0263–0266) was reported (Pereira *et al.*, 2008). Although a role in electron transfer from periplasmic oxidations or from reduced menaquinones to sulfate would seem likely for this complex, no experimental evidence supported this possibility until now. Furthermore, many other genes from the *c*-type cytochrome network were differentially expressed in the sulfidogenic metabolism: cytochrome *c* family proteins

Table 3. RT-PCR confirmation of genes differentially expressed in *D. vulgaris* under different experimental conditions

–, No change.

Gene ID	Description	Sulfidogenic vs syntrophic	
		Microarray	RT-PCR
DVU0148	Lipoprotein, putative	−3.10	−2.53
DVU0263	Acidic cytochrome c_3	1.50	1.65
DVU0402	Dissimilatory sulfite reductase alpha subunit (*dsrA*)	–	–
DVU0431	Ech hydrogenase, subunit EchD, putative	1.13	3.22
DVU0533	*hmc* operon protein 4	−1.27	−1.79
DVU0777	ATP synthase, F_1 alpha subunit (*atpA*)	1.24	2.15
DVU0778	ATP synthase, F_1 delta subunit (*atpH*)	1.46	2.22
DVU0847	Adenylyl-sulphate reductase, alpha subunit (*aprA*)	–	–
DVU2108	MTH1175-like domain family protein	2.15	2.56
DVU2571	Ferrous iron transport protein B (*feoB*)	1.62	1.95

(DVU0922, DVU3107 and DVU 3144) and cytochrome *c* oxidase (DVU1812) were upregulated, and others were downregulated (DVU0702, DVU2484 and DVU3171). Genes of the cytochrome *d* ubiquinol oxidase subunits I and II were both upregulated (DVU3270 and DVU3271; Supplementary Table S1). Together, these results show that a significant part of the *c*-type cytochrome network was upregulated during the *D. vulgaris* metabolic change from syntrophic to sulfidogenic, suggesting that part of the *c*-type cytochrome network is not involved in the transfer of electrons during *D. vulgaris* syntrophic metabolism. The microarray result for DVU0263 was also confirmed by qRT-PCR (Table 3). Although the tetrahaemic cytochrome c_3 (DVU3171) is generally regarded as the primary electron acceptor from periplasmic hydrogen oxidation and accounts for the majority of the *c*-type cytochromes of the periplasm (Aubert *et al.*, 2000), no upregulation of gene expression was found for DVU3171 during the sulfidogenic metabolism, suggesting that it may be constitutively expressed under these conditions.

It was anticipated that genes encoding adenylyl-sulfate reductase (*aprAB*) and dissimilatory sulfite reductase (*dsrAB*) would be upregulated in *D. vulgaris* after the addition of sulfate. Surprisingly, these genes were not upregulated by the sulfate perturbation, and the results were confirmed by qRT-PCR analysis (Table 3). This suggests that these genes are constitutively expressed in *D. vulgaris* during both syntrophic and sulfidogenic metabolism. Nevertheless, the microarray data showed that the DsrMKJOP operon (DVU1286–1290), which is thought to donate electrons to DsrAB, was upregulated (Haveman *et al.*, 2004) (Table 2). Constitutive expression of key genes involved in sulfate reduction in *D. vulgaris* may point to a preference for the sulfidogenic metabolism: the moment sulfate is present, *D. vulgaris* is able to use it as a terminal electron acceptor. In an early work on *Desulfobacterium autotrophicum*, real-time RT-PCR was used to determine *dsr* expression relative to the amount of 16S rRNA under different growth conditions during the transition from exponential to stationary phase: sulphate respiration with lactate, thiosulphate respiration with lactate, sulphate respiration with H_2, and pyruvate fermentation. The results showed that although *dsr* is expressed constitutively under all conditions, DSR mRNA content per cell varies under different growth conditions (Neretin *et al.*, 2003).

Cell envelope processes responsive to the lifestyle change

Six genes involved in cell envelope processes were downregulated directly after perturbing the syntrophic cultures with sulfate, and remained downregulated in the sulfidogenic phase, indicating their role during syntrophic growth of *D. vulgaris* on lactate. These included two genes encoding membrane proteins, three genes encoding lipoproteins and one gene encoding a UDP-*N*-acetylmuramoyl-L-alanine-D-glutamate ligase (Table 2). Three genes involved in cell envelope processes were upregulated directly after perturbing the syntrophic dual-cultures with sulfate and remained upregulated in the sulfidogenic phase, indicating their role in the *D. vulgaris* sulfidogenic metabolism. These genes included two genes encoding putative lipoproteins and one gene encoding a putative membrane protein (Table 2). Other genes involved in cell envelope processes were also down- or upregulated after the perturbation with sulfate, but these responsive genes were only observed directly after the perturbation or during the sulfidogenic metabolism (Supplementary Table S2).

The most significant finding from the microarray data was the decline in expression level of a three-gene cluster associated with the *D. vulgaris* switch from syntrophic to sulfidogenic metabolism (Table 2). DVU0148, DVU0149 and DVU0150 encode lipo- and membrane proteins and were expressed at 1.4–3.1-fold lower levels during the perturbation and subsequent sulfidogenic metabolism. The genome sequence shows these three genes are in the same operon as two other genes (DVU0146 and DVU0147) (Dehal *et al.*, 2010). Downstream of this five-gene cluster is a

gene encoding a response regulator (DVU0145). Remarkably, this five-gene cluster has gene orthologues and an almost identical organization only in another syntrophic bacterium, *Syntrophobacter fumaroxidans* (Harmsen *et al.*, 1998), and four recently sequenced *Desulfovibrio* strains (*D. vulgaris* strain DP4; *D. vulgaris* strain Miyazaki; *Desulfovibrio piger* and *Desulfovibrio desulfuricans*) (Fig. 2). Orthologues of DVU0145 are not found in *Desulfovibrio desulfuricans* and *S. fumaroxidans*, although in *D. desulfuricans* there is a gene encoding a putative histidine kinase HAMP region domain protein (Ddes_1643) upstream of the five-gene cluster, and in *S. fumaroxidans* there are two genes encoding a histidine kinase and a response regulator upstream of the five-gene cluster (Sfum_0622 and Sfum_0623, respectively) (Fig. 2). The DVU0149 gene was expressed at twofold lower levels in the sulfidogenic relative to the syntrophic metabolism. This gene encodes a protein of unknown function from the DUF81-like domain membrane protein family with gene orthologues in several other bacteria and archaea. Interestingly, multiple copies (greater than three) of DVU0149 and its homologue Sfum_626 are found in the genomes of both *D. vulgaris* and *S. fumaroxidans* (data not shown). Regulon prediction (based on http://www.microbesonline.org/) suggested that this gene cluster forms a membrane complex of unknown function (Fig. 2). Downregulation of DVU0148 was also confirmed by qRT-PCR (Table 3). To further confirm the idea that these three genes are involved in the syntrophic metabolism of *D. vulgaris* we also determined their expression patterns when *D. vulgaris* had been grown as a sulfidogen in pure culture under various conditions. None of the pure culture conditions showed gene expression changes for this gene cluster (Zhang *et al.*, 2006a, b). These results provided further support for our hypothesis that these three genes are involved in the *D. vulgaris* syntrophic metabolism.

Other genes responsive to the lifestyle change

Two genes encoding iron transport and binding proteins were upregulated by sulfate perturbation and remained upregulated in the sulfidogenic metabolism, indicating their role during sulfidogenic growth of *D. vulgaris* on lactate (Table 2). These genes encode different components of a ferrous (Fe^{2+}) iron transport complex and were expressed at 1.1–2.0-fold higher levels in the microarray, which was also confirmed by qRT-PCR results (Table 3). One other transport and binding protein gene encoding a component of an Fe^{2+} transport complex was also upregulated in the sulfidogenic metabolism. Under anaerobic conditions, Fe^{2+} predominates over ferric iron, and can be transported by the ATP-dependent ferrous iron transport system FeoAB. Genomes of anaerobic δ-proteobacteria typically contain multiple copies of the *feoAB* genes, and in general lack ABC transporters for siderophores. Furthermore, regulation of iron metabolism in bacteria is mediated by the ferric-uptake regulator protein (FUR), which represses transcription upon interaction with

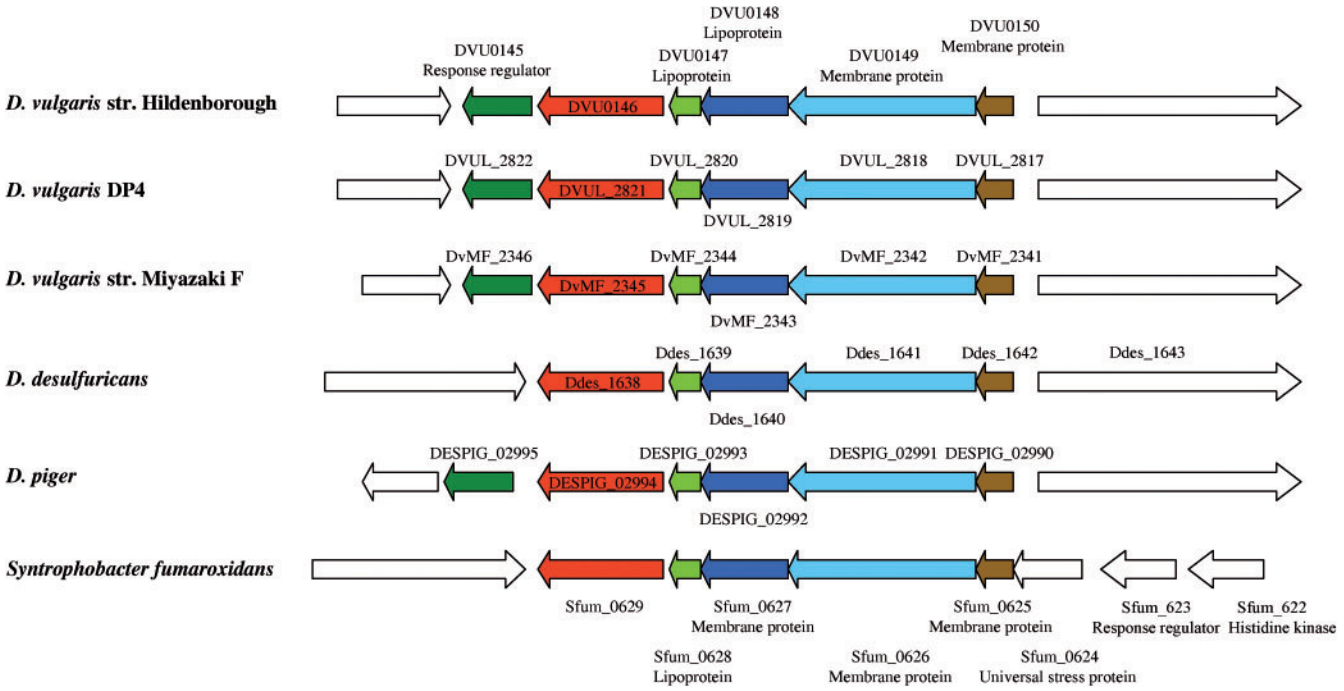

Fig. 2. Schematic diagram of a *D. vulgaris* strain Hildenborough gene cluster together with its orthologues in *S. fumaroxidans* and four recently sequenced *Desulfovibrio* strains. The cluster was significantly downregulated during the shift from the syntrophic to the sulfate-reducing lifestyle.

ferrous ions (Rodionov *et al.*, 2004). Our results suggested that *D. vulgaris* started to take up iron by a Fe^{2+} transport system (*feoAB*) after it switched its metabolism from syntrophic to sulfidogenic. The sequence of the FeoB protein revealed regions of homology to ATPases, which implies that Fe^{2+} uptake by *D. vulgaris* is ATP driven (Kammler *et al.*, 1993). Iron serves as an essential component of haem and iron–sulfur centres in a variety of enzymes, including *c*-type cytochromes, hydrogenases and ferredoxins. In fact, genes for a number of these enzymes were upregulated during the *D. vulgaris* sulfidogenic metabolism (Table 2), suggesting that there is a genuine requirement for Fe^{2+}. It seems that for the fulfilment of this prerequisite for Fe^{2+} an active uptake mechanism would be required.

While this paper was in preparation, a similar study was published on the electron transfer system of *D. vulgaris* when it is grown syntrophically with *Methanococcus maripaludis* (Walker *et al.*, 2009). There are four major differences in experimental design between the two studies: the methanogenic partner in Walker *et al.* (2009) was *Methanococcus maripaludis*, whereas we used *M. barkeri*; the *D. vulgaris* and *Methanococcus maripaludis* dual-culture and the *D. vulgaris* monoculture were cultivated in parallel in different chemostats by Walker *et al.* (2009), while we performed a perturbation experiment by adding sulfate to the chemostat co-culture to produce the *D. vulgaris* sulfidogenic metabolism; the sulfidogenic monoculture of Walker *et al.* (2009) was sulfate-limited, whereas our *D. vulgaris* sulfidogenic metabolism was lactate-limited; and the cell ratio (between *D. vulgaris* and *Methanococcus maripaludis*) during steady-state dual-culture growth was higher (4 : 1) in the study of Walker and co-workers compared with the 1 : 1 (*D. vulgaris* to *M. barkeri*) in our study. Nevertheless, some similar results were obtained. These included the upregulation during the *D. vulgaris* syntrophic metabolism of the high-molecular-mass cytochrome complex (DVU0533, encoding Hmc protein 4), the DVU0145–0150 cellular membrane gene cluster of unknown function and heterodisulfide reductase (*hdrAB*), and the downregulation of genes involved in iron transport (*feoB* and *feoA*) (Walker *et al.*, 2009). Our study thus provides further confirmation that these genes are an important part of metabolism during the syntrophic growth of *D. vulgaris*. However, no change in gene expression was observed in our study for cytoplasmic hydrogenase *Coo*, periplasmic hydrogenases *hydAB* and hydrogenases *hynAB*-1, which were found to be upregulated in syntrophic growth by Walker *et al.* (2009). These and other differences could reasonably be the result of the four quite substantial differences noted above.

The global description and functional interpretation of the transcriptomics responses of *D. vulgaris* as a member of a *D. vulgaris–M. barkeri* syntrophic dual-culture provide a broader foundation, along with the results of Walker and co-workers, for understanding the metabolic mechanisms and molecular regulation of *Desulfovibrio*–methanogen syntrophic interactions.

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REFERENCES

Albert, T. J., Norton, J., Ott, M., Richmond, T., Nuwaysir, K., Nuwaysir, E. F., Stengele, K. P. & Green, R. D. (2003). Light directed 5′→3′ synthesis of complex oligonucleotide microarrays. *Nucleic Acids Res* **31**, e35.

Aubert, C., Brugna, M., Dolla, A., Bruschi, M. & Giudici-Orticoni, M. T. (2000). A sequential electron transfer from hydrogenases to cytochromes in sulfate-reducing bacteria. *Biochim Biophys Acta* **1476**, 85–92.

Bender, K. S., Yen, H. C., Hemme, C. L., Yang, Z., He, Z., He, Q., Zhou, J., Huang, K. H., Alm, E. J. & other authors (2007). Analysis of a ferric uptake regulator (*Fur*) mutant of *Desulfovibrio vulgaris* Hildenborough. *Appl Environ Microbiol* **73**, 5389–5400.

Benjemini, Y. & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol* **57**, 289–300.

Bryant, M. P., Wolin, E. A., Wolin, M. J. & Wolfe, R. S. (1967). *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. *Arch Mikrobiol* **59**, 20–31.

Bryant, M. P., Campbell, L. L., Reddy, C. A. & Crabill, M. R. (1977). Growth of *Desulfovibrio* in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. *Appl Environ Microbiol* **33**, 1162–1169.

Chhabra, S. R., He, Q., Huang, K. H., Gaucher, S. P., Alm, E. J., He, Z., Hadi, M. Z., Hazen, T. C., Wall, J. D. & other authors (2006). Global analysis of heat shock response in *Desulfovibrio vulgaris* Hildenborough. *J Bacteriol* **188**, 1817–1828.

Clark, M. E., He, Q., He, Z., Huang, K. H., Alm, E. J., Wan, X. F., Hazen, T. C., Arkin, A. P., Wall, J. D. & other authors (2006). Temporal transcriptomic analysis as *Desulfovibrio vulgaris* Hildenborough transitions into stationary phase during electron donor depletion. *Appl Environ Microbiol* **72**, 5578–5588.

Culley, D. E., Kovacik, W. P., Jr, Brockman, F. J. & Zhang, W. (2006). Optimization of RNA isolation from the archaebacterium *Methanosarcina barkeri* and validation for oligonucleotide microarray analysis. *J Microbiol Methods* **67**, 36–43.

Cypionka, H. (2000). Oxygen respiration in *Desulfovibrio* species. *Annu Rev Microbiol* **54**, 827–848.

Dehal, P. S., Joachimiak, M. P., Price, M. N., Bates, J. T., Baumohl, J. K., Chivian, D., Friedland, G. D., Huang, K. H., Keller, K. & other authors (2010). MicrobesOnline: an integrated portal for comparative and functional genomics. *Nucleic Acids Res* **38**, D396–D400.

Harmsen, H. J., Van Kuijk, B. L., Plugge, C. M., Akkermans, A. D., De Vos, W. M. & Stams, A. J. (1998). *Syntrophobacter fumaroxidans* sp. nov., a syntrophic propionate-degrading sulfate-reducing bacterium. *Int J Syst Bacteriol* **48**, 1383–1387.

Haveman, S. A., Greene, E. A., Stilwell, C. P., Voordouw, J. K. & Voordouw, G. (2004). Physiological and gene expression analysis of inhibition of *Desulfovibrio vulgaris* Hildenborough by nitrite. *J Bacteriol* **186**, 7944–7950.

He, Q., Huang, K. H., He, Z., Alm, E. J., Fields, M. W., Hazen, T. C., Arkin, A. P., Wall, J. D. & Zhou, J. (2006). Energetic consequences of nitrite stress in *Desulfovibrio vulgaris* Hildenborough inferred from global transcriptional analysis. *Appl Environ Microbiol* **72**, 4370–4381.

Heidelberg, J. F., Seshadri, R., Haveman, S. A., Hemme, C. L., Paulsen, I. T., Kolonay, J. F., Eisen, J. A., Ward, N., Methe, B. & other authors (2004). The genome sequence of the anaerobic sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough. *Nat Biotechnol* **22**, 554–559.

Holmer, M. & Kristensen, E. (1994). Co-existence of sulfate reduction and methane production in an organic-rich sediment. *Mar Ecol Prog Ser* **107**, 177–184.

Kammler, M., Schön, C. & Hantke, K. (1993). Characterization of the ferrous iron uptake system of *Escherichia coli*. *J Bacteriol* **175**, 6212–6219.

Kuivila, K.M., Murray, J. W. & Devol, A. H. (1990). Methane production in the sulfate depleted sediments of two marine basins. *Geochim Cosmochim Acta* **54**, 403–411.

Leloup, J., Fossing, H., Kohls, K., Holmkvist, L. & Jørgensen, B. B. (2009). Sulfate-reducing bacteria in marine sediment (Aarhus Bay, Denmark): abundance and diversity related to geochemical zonation. *Environ Microbiol* **11**, 1278–1291.

Livak, K. J. & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**, 402–408.

McInerney, M. J., Mackie, R. I. & Bryant, M. P. (1981). Syntrophic association of a butyrate-degrading bacterium and *Methanosarcina* enriched from bovine rumen fluid. *Appl Environ Microbiol* **41**, 826–828.

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**, 325–335.

Meuer, J., Kuettner, H. C., Zhang, J. K., Hedderich, R. & Metcalf, W. W. (2002). Genetic analysis of the archaeon *Methanosarcina barkeri* Fusaro reveals a central role for Ech hydrogenase and ferredoxin in methanogenesis and carbon fixation. *Proc Natl Acad Sci U S A* **99**, 5632–5637.

Mukhopadhyay, A., He, Z., Alm, E. J., Arkin, A. P., Baidoo, E. E., Borglin, S. C., Chen, W., Hazen, T. C., He, Q. & other authors (2006). Salt stress in *Desulfovibrio vulgaris* Hildenborough: an integrated genomics approach. *J Bacteriol* **188**, 4068–4078.

Mukhopadhyay, A., Redding, A. M., Joachimiak, M. P., Arkin, A. P., Borglin, S. E., Dehal, P. S., Chakraborty, R., Geller, J. T., Hazen, T. C. & other authors (2007). Cell-wide responses to low-oxygen exposure in *Desulfovibrio vulgaris* Hildenborough. *J Bacteriol* **189**, 5996–6010.

Muyzer, G. & Stams, A. J. M. (2008). The ecology and biotechnology of sulphate-reducing bacteria. *Nat Rev Microbiol* **6**, 441–454.

Neretin, L. N., Schippers, A., Pernthaler, A., Hamann, K., Amann, R. & Jørgensen, B. B. (2003). Quantification of dissimilatory (bi)sulphite reductase gene expression in *Desulfobacterium autotrophicum* using real-time RT-PCR. *Environ Microbiol* **5**, 660–671.

Nuwaysir, E. F., Huang, W., Albert, T. J., Singh, J., Nuwaysir, K., Pitas, A., Richmond, T., Gorski, T., Berg, J. P. & other authors (2002). Gene expression analysis using oligonucleotide arrays produced by maskless photolithography. *Genome Res* **12**, 1749–1755.

Odom, J. M. & Peck, H. D., Jr (1981). Hydrogen cycling as a general mechanism for energy coupling in the sulfate-reducing bacteria *Desulfovibrio* sp. *FEMS Microbiol Lett* **12**, 47–50.

Oremland, R. S. & Polcin, S. (1982). Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments. *Appl Environ Microbiol* **44**, 1270–1276.

Peck, H. D., Jr (1966). Phosphorylation coupled with electron transfer in extracts of the sulfate reducing bacterium *Desulfovibrio gigas*. *Biochem Biophys Res Commun* **22**, 112–118.

Pereira, P. M., He, Q., Valente, F. M. A., Xavier, A. V., Zhou, J., Pereira, I. A. C. & Louro, R. O. (2008). Energy metabolism in *Desulfovibrio vulgaris* Hildenborough: insights from transcriptome analysis. *Antonie van Leeuwenhoek* **93**, 347–362.

Rodionov, D. A., Dubchak, I., Arkin, A., Alm, E. & Gelfand, M. S. (2004). Reconstruction of regulatory and metabolic pathways in metal-reducing delta-proteobacteria. *Genome Biol* **5**, R90.

Rodrigues, R., Valente, F. M., Pereira, I. A., Oliveira, S. & Rodrigues-Pousada, C. (2003). A novel membrane-bound Ech [NiFe] hydrogenase in *Desulfovibrio gigas*. *Biochem Biophys Res Commun* **306**, 366–375.

Schink, B. (1997). Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Biol Rev* **61**, 262–280.

Schink, B. (2002). Synergistic interactions in the microbial world. *Antonie van Leeuwenhoek* **81**, 257–261.

Scholten, J. C. & Conrad, R. (2000). Energetics of syntrophic propionate oxidation in defined batch and chemostat cocultures. *Appl Environ Microbiol* **66**, 2934–2942.

Scholten, J. C., Culley, D. E., Brockman, F. J., Wu, G. & Zhang, W. (2007a). Evolution of the syntrophic interaction between *Desulfovibrio vulgaris* and *Methanosarcina barkeri*: Involvement of an ancient horizontal gene transfer. *Biochem Biophys Res Commun* **352**, 48–54.

Scholten, J. C., Culley, D. E., Nie, L., Munn, K. J., Chow, L., Brockman, F. J. & Zhang, W. (2007b). Development and assessment of whole-genome oligonucleotide microarrays to analyze an anaerobic microbial community and its responses to oxidative stress. *Biochem Biophys Res Commun* **358**, 571–577.

Simon, R. M., Korn, E. L., McShane, L. M., Radmacher, M. D., Wright, G. E. & Zhao, Y. (2003). *Design and Analysis of DNA Microarray Investigations*. New York: Springer.

Stams, A. J. M. (1994). Metabolic interactions between anaerobic bacteria in methanogenic environments. *Antonie van Leeuwenhoek* **66**, 271–294.

Stams, A. J. M. & Plugge, C. M. (2009). Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nat Rev Microbiol* **7**, 568–577.

Tang, Y., Pingitore, F., Mukhopadhyay, A., Phan, R., Hazen, T. C. & Keasling, J. D. (2007). Pathway confirmation and flux analysis of central metabolic pathways in *Desulfovibrio vulgaris* Hildenborough using gas chromatography-mass spectrometry and Fourier transform ion cyclotron resonance mass spectrometry. *J Bacteriol* **189**, 940–949.

Vignais, P. M., Billoud, B. & Meyer, J. (2001). Classification and phylogeny of hydrogenases. *FEMS Microbiol Rev* **25**, 455–501.

Voordouw, G. (1995). The genus *Desulfovibrio*: the centennial. *Appl Environ Microbiol* **61**, 2813–2819.

Walker, C. B., He, Z., Yang, Z. K., Ringbauer, J. A., Jr, He, Q., Zhou, J., Voordouw, G., Wall, J. D., Arkin, A. P. & other authors (2009). The electron transfer system of syntrophically grown *Desulfovibrio vulgaris*. *J Bacteriol* **191**, 5793–5801.

Widdel, F. & Hansen, T. A. (1991). The dissimilatory sulphate and sulphur-reducing bacteria. In *The Prokaryotes*, 2nd edn, vol. I, pp. 583–624. Edited by A. Balows, H. G. Truper, M. Dworkin, W. Harder & K. H. Schleiter. New York: Springer.

Winfrey, M. R. & Ward, D. M. (1983). Substrates for sulfate reduction and methane production in intertidal sediments. *Appl Environ Microbiol* **45**, 193–199.

Zhang, W., Culley, D. E., Scholten, J. C., Hogan, M., Vitiritti, L. & Brockman, F. J. (2006a). Global transcriptomic analysis of *Desulfovibrio vulgaris* on different electron donors. *Antonie van Leeuwenhoek* **89**, 221–237.

Zhang, W., Culley, D. E., Hogan, M., Vitiritti, L. & Brockman, F. J. (2006b). Oxidative stress and heat-shock responses in *Desulfovibrio vulgaris* by genome-wide transcriptomic analysis. *Antonie van Leeuwenhoek* **90**, 41–55.

Zhang, W., Gritsenko, M. A., Moore, R. J., Culley, D. E., Nie, L., Petritis, K., Strittmatter, E., Camp, D. G., II, Smith, R. D. & Brockman, F. J. (2006c). A proteomic view of *Desulfovibrio vulgaris* metabolism as determined by liquid chromatography coupled with tandem mass spectrometry. *Proteomics* **6**, 4286–4299.

Zhang, W., Culley, D. E., Nie, L. & Brockman, F. J. (2006d). DNA microarray analysis of anaerobic *Methanosarcina barkeri* reveals responses to heat shock and air exposure. *J Ind Microbiol Biotechnol* **33**, 784–790.

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