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.

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 CO₂ (Muyzer & Stams, 2008). Where sediments are high in organic matter, sulfate is depleted at shallow sediment depths and biogenic methane production results.
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; McNerney 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 H2 (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 transcripational 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 H2 plus CO2 (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 (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 log₂ 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 (Benjeminini & 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...
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...
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 (Heidelberger *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; Heidelberger *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...
in the transfer of electrons during the \textit{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 2. Key responsive genes in \textit{D. vulgaris} following lifestyle changes

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<td>Sulfidogenic vs syntrophic</td>
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<td>DVU0173</td>
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<td>DNA binding response regulator</td>
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<tr>
<td>DVU0260</td>
<td>Response regulator</td>
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<td>DVU0261</td>
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<td>DVU0263</td>
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<td>Ech hydrogenase, subunit EchA, putative</td>
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### Cell envelope

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### Iron transport and binding proteins

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

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<td>DVU0148</td>
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<td>DVU0402</td>
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</tbody>
</table>

(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 DVU3272; 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 c₃ (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-sulphate reductase (aprAB) and dissipimilatory 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 Desulfovibrio 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 H₂, 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

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**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 fulfillment 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.arkeri*; 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.arkeri*) 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.arkeri* 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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