Identification of proteins and genes expressed by *Methylophaga thiooxydans* during growth on dimethylsulfide and their presence in other members of the genus

Eileen Kröber[1,2], Hendrik Schäfer[1]*

[1] School of Life Sciences, Gibbet Hill Campus, University of Warwick, Coventry, CV4 7AL, United Kingdom

[2] Current address: Microbial Biogeochemistry, RA Landscape Functioning, ZALF Leibniz Centre for Agricultural Landscape Research, Müncheberg, Germany

*Correspondence to

H Schäfer, School of Life Sciences, Gibbet Hill Campus, University of Warwick, Coventry, CV4 7AL, United Kingdom

Abstract

Dimethylsulfide is a volatile organic sulfur compound that provides the largest input of biogenic sulfur from the oceans to the atmosphere, and thence back to land, constituting an important link in the global sulfur cycle. Microorganisms degrading DMS affect fluxes of DMS in the environment, but the underlying metabolic pathways are still poorly understood. *Methylophaga thiooxydans* is a marine methylotrophic bacterium capable of growth on DMS as sole source of carbon and energy. Using proteomics and transcriptomics we identified genes expressed during growth on dimethylsulfide and methanol to refine our knowledge of the metabolic pathways that are involved in DMS and methanol degradation in this strain. Amongst the most highly expressed genes on DMS were the two methanethiol oxidases driving the oxidation of this reactive and toxic intermediate of DMS metabolism. Growth on DMS also increased expression of the enzymes of the tetrahydrofolate linked pathway of formaldehyde oxidation, in addition to the tetrahydromethanopterin linked pathway. Key enzymes of the inorganic sulfur oxidation pathway included flavocytochrome *c* sulfide dehydrogenase, sulfide quinone oxidoreductase, and persulfide dioxygenases. A *sulP* permease was also expressed during growth on DMS. Proteomics and transcriptomics also identified a number of highly expressed proteins and gene products whose function is currently not understood. As the identity of some enzymes of organic and inorganic sulfur metabolism previously detected in *Methylophaga* has not been characterised at the genetic level yet, highly expressed uncharacterised genes provide new targets for further biochemical and genetic analysis. A pan-genome analysis of six available *Methylophaga* genomes showed that only two of the six investigated strains, *M. thiooxydans* and *M. sulfidovorans* have the gene encoding methanethiol oxidase, suggesting that growth on methylated sulfur compounds of *M. aminisulfidivorans* is likely to involve different enzymes and metabolic intermediates. Hence, the pathways of DMS-utilization and subsequent C_1 and sulfur oxidation are not conserved across *Methylophaga* isolates that degrade methylated sulfur compounds.

Introduction

Dimethylsulfide is a volatile methylated sulfur compound that has been associated with the 'smell of the sea' (Stiefel, 1996) and which plays a crucial role in the global sulfur cycle (Lomans et al., 2002). The observation of significant concentrations of DMS in the marine boundary layer (Lovelock et al., 1972) led to the realisation that marine emissions of DMS are driving transfer of sulfur between the marine and terrestrial environment via the atmosphere. In addition, its atmospheric breakdown products (mainly sulfate, sulfur dioxide and methanesulfonic acid) are important precursors for secondary organic aerosols. These play a role in climate feedbacks by reflecting solar radiation from the sun back to space and serving as cloud condensation nuclei (CCN), which support the formation of clouds that reflect further sunlight and thus may contribute to climate regulation (Charlson et al., 1987;Simó, 2001;Carslaw et al., 2010;Lana et al., 2011).

The marine environment represents the largest source of DMS to the atmosphere (Watts, 2000) and the amount of DMS available for sea-to-air transfer depends on a number of microbial pathways that lead to production of DMS and its degradation in the surface ocean (Schäfer et al., 2010;Curson et al., 2011;Lidbury et al., 2016a;Curson et al., 2018). Bacterial degradation of DMS is a major sink for DMS (Kiene and Bates, 1990) and a number of methylotrophic marine bacteria that are able to grow using DMS as a sole source of carbon and energy have been described previously, some of these belong to the genus *Methylophaga* (Janvier and Grimont, 1995). *Methylophaga* species able to grow on methylated sulfur compounds include the restricted facultative species *M. sulfidovorans* (De Zwart et al., 1997), *M. aminisulfidivorans* (Kim et al., 2007) and *M. thiooxydans* (Boden et al., 2010). It was shown that the latter produces tetrathionate as end-product of its sulfur metabolism (Boden et al., 2010). In addition to DMS, *M. thiooxydans* can grow on the one-carbon compounds methanol, dimethylamine (DMA), trimethylamine (TMA), as well as on fructose as sole carbon source (Boden et al., 2010). Boden et al. (2010) characterized the DMS-degradation pathway in *M. thiooxydans* based on enzyme assays and analysis of sulfur intermediates and end products and compared it to *M. thiooxydans* grown on methanol. Methanol dehydrogenase activity was detected in biomass grown on methanol and DMS; however, no enzyme activities were detected for degradation of DMS and methanethiol, an intermediate of DMS degradation, during growth on methanol (Boden et al., 2010). Furthermore, during growth on DMS, DMS monooxygenase (De Bont et al., 1981;Boden et al., 2010) activity was not detected, and the presence of a methyltransferase (Visscher and Taylor, 1993), which could be responsible for the initial step of DMS degradation, was suggested (Boden et al., 2010) although this 'DMS-methyltransferase' was not identified.

The aim of this study was to further characterise the metabolic pathways contributing to DMS degradation in *Methylophaga thiooxydans*. Proteomics and transcriptomics analyses were carried out with *Methylophaga thiooxydans* DMS010 in order to identify enzymes involved in DMS degradation in this model organism and potentially identify candidates of thus-far unidentified enzymes for further characterisation.

Materials and Methods

Cultivation of *Methylophaga thiooxydans*

Methylophaga thiooxydans DMS010 was grown at 25°C in triplicate cultures using sterile marine ammonium mineral salts (MAMS) medium (Thompson et al., 1995), supplemented either with 1 mM methanol or 1 mM DMS as a sole carbon source and a 10% (v/v) inoculum. The cultures were monitored twice daily by measurement of optical density at 560 nm (OD560) in an UltrospecTM 3100 pro spectrophotometer (Amersham Biosciences Corp., New Jersey, USA). Quantitative determination of DMS was as described previously (Lidbury et al., 2016a). Cells were harvested at an OD560 of approximately 0.4 (exponential growth phase) by centrifugation at 13,000 × *g* for 30 minutes at 4°C. The supernatant was discarded, and cells were washed and resuspended in PIPES buffer (1,4-piperazinediethanesulfonic acid, pH 7.8) or TRIzol® reagent (Life Technologies Corporation, Carlsbad, USA) for proteomics or transcriptomics, respectively. Cells were then snap-frozen in liquid nitrogen and stored at -80°C until further processing.

Proteomics

Protein extraction and quantification

For proteomics, proteins were extracted from triplicate *Methylophaga thiooxydans* DMS010 cell culture pellets resuspended in PIPES buffer. The homogeneous cell suspension was centrifuged at 9855 x *g* for 20 minutes at 4°C. The supernatant was discarded and the pellet was resuspended in 1 mL of an ice-cooled PIPES buffer with addition of 160 µg mL^{-1} benzamidine and 1 µg DNase was added. The PIPES buffer with 160 µg mL^{-1} benzamidine was prepared by mixing 0.39 g of benzamidine hydrochloride hydrate (98%) (Sigma-Aldrich) with 5 mL water. 0.2 mL of this solution was then mixed with 100 mL PIPES buffer and cooled on ice. Another 2 mL of this PIPES buffer (160µg ml^{-1} benzamidine) was added and the cells were broken by three passages through a French pressure cell (American Instrument Corporation, Hartland, USA) at 1000 psi. Cell debris were removed by centrifugation at 9855 x *g* for 20 minutes at 4°C. The supernatant was centrifuged again at 106,934 x *g* for 45 minutes at 4°C. After centrifugation the supernatant was transferred into 4.5 mL tubes (soluble protein fraction).

The pellet was resuspended in 4.5 mL PIPES buffer (pH 7.8) and centrifuged again at 106,934 x *g* for 45 minutes at 4°C. Pellet resuspension and centrifugation was repeated, the supernatant was discarded and the pellet was resuspended in 500 µL PIPES buffer (without benzamidine). Protein concentrations were determined using the Bradford protein assay (Bradford, 1976) and samples of the protein preparations were checked via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a precast Mini-PROTEAN® gel (Bio-Rad Laboratories Inc.) and a Mini-PROTEAN® Tetra cell (Bio-Rad Laboratories Inc.). About 15 µg of protein was loaded onto the gel per sample and 20 µL of Precision Plus ProteinTM Standard (Dual Color, Bio-Rad Laboratories Inc.). The gel was run at 200V for about 35 to 40 minutes. Gels were stained overnight with InstantBlueTM protein stain (Sigma-Aldrich). SDS-PAGE analysis of soluble and membrane protein fractions showed reproducible protein profiles of replicates within each fraction type and carbon source but revealed different profiles of soluble and membrane fraction between carbon sources (Supplementary Figure S1).

Protein identification and data analysis

The soluble protein fractions of *Methylophaga thiooxydans* DMS010 grown either on DMS or methanol (triplicates) were submitted for proteomic analysis to the Proteomics facility of the School of Life Sciences at the University of Warwick. The samples were subjected to a tryptic digest (Lidbury et al., 2016b) followed by high-resolution mass spectrometry analysis using the Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher, Bremen, Germany). Peptide sequences obtained from MS/MS spectra were searched against *Methylophaga thiooxydans* DMS010 peptide sequences (database) using Mascot (Matrix Science Inc., Boston, USA) in order to identify proteins. Visualization and validation of the peptides was done with Scaffold (Proteome Software, Inc., Portland, USA). Identified peptides and proteins were searched and placed into a pathway map using KEGG, Swiss-Prot, ExPASy and UniProt. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD011992 and 10.6019/PXD011992 (http://www.proteomexchange.org).

Transcriptomics

RNA extraction and quantification

RNA was extracted from *Methylophaga thiooxydans* cell pellets resuspended in TRIzol® reagent following the manufacturer's instructions. Quantification of RNA was performed using the ND-1000 spectrophotometer (NanoDrop Technologies Inc.). In addition, the concentration and purity of the RNA was also determined on a Bioanalyzer (Agilent 2100 Bioanalyzer,

Agilent Technologies, Inc., USA) using an mRNA Nano chip (Agilent, Supplementary Figure S5).

rRNA depletion

Depletion of rRNA was carried out using the Ribo-ZeroTM Magnetic kit for bacteria (Epicentre, Madison, USA) following the manufacturer's instructions. Enrichment of mRNA was checked on the Agilent 2100 Bioanalyzer using an mRNA Nano chip (Agilent).

Library preparation and RNA sequencing

Following rRNA depletion, samples were submitted to the Genomics facility of the School of Life Sciences at the University of Warwick for library preparation and RNA-sequencing (Illumina Hi-Seq 4000). The Genomics facility at the University of Warwick provided raw fastq reads.

RNA sequencing data analysis

RNA sequencing reads were analysed using Rockhopper (McClure et al., 2013). The reference-based transcriptome analysis was carried out by aligning the reads to a *Methylophaga thiooxydans* DMS010 reference genome which was constructed on the Rapid Annotation Server (RAST) using scaffolds available from the National Center for Biotechnology Information (NCBI) (ASM15635v1). Then data was normalized in Rockhopper in order to allow for data comparison between DMS-grown and methanol-grown *Methylophaga thiooxydans* DMS010 cultures. Afterwards, transcripts were assembled, transcript boundaries were identified and transcript abundance was quantified. Finally, a test for differential gene expression was carried out in Rockhopper. Rockhopper analyses the data by aligning the reads to the *Methylophaga thiooxydans* DMS010 genome. Normalisation between the triplicate experiments and different conditions (DMS/methanol) is done by upper quartile normalization. The transcripts are assembled and transcript boundaries are identified. Quantification of transcript abundance is done in RPKM (reads per kilobase per million mapped reads). RPKM is a common measure for counting gene expression and sums the number of reads for a gene and divides by the gene's length and the total number of reads (McClure et al., 2013). Rockhopper reports the expression level, calculated from the triplicates, using RPKM. However, instead of dividing by the total number of reads, Rockhopper divides by the upper quartile of gene expression which has been suggested as a more robust normalization method (McClure et al., 2013) . RNAseq data have been deposited in the NCBI Short Read Archive under accession number PRJNA509071.

A comparison between proteomics and transcriptomics was done by mapping the expression data of both approaches onto the *M. thiooxydans* genome using the CGView comparison tool using an in-house pipeline by Paul Stothard (Grant et al., 2012).

Pan-genome analysis

Genome data acquisition

Six *Methylophaga* genomes (*M. thiooxydans* DMS0101, *M. sulfidovorans* DSM11578, *M. aminisulfidovorans*, *M. lonarensis*, *M. nitratireducenticrescens* strain JAM1, *M. frappieri* strain JAM7) available through the Integrated Microbial Genomes (IMG) database (https://img.jgi.doe.gov/) were used for comparative genome analysis (Markowitz et al., 2013). Accession numbers and additional genome characteristics are listed in Supplementary Table S1.

Pan-genome analysis including average amino acid identity (AAI) analysis, pan-genome tree construction and determination of core, dispensable genes and singletons (unique genes) was carried out using the Efficient Database framework for comparative Genome Analyses using BLAST score Ratios (EDGAR) platform (Blom et al., 2016).

In order to compare the genetic potential for dimethylsulfide degradation and sulfur oxidation within the available *Methylophaga* genomes, known protein sequences involved in dimethylsulfide degradation and sulfur oxidation pathways were used as query sequences through the BLAST (blastp) program (Altschul et al., 1990) available within the Rapid Annotation using Subsystem Technology (RAST) server (Aziz et al., 2008). The list of protein queries used is given in Supplementary Table S2.

Results

Known genes in *Methylophaga thiooxydans* genome with potential roles in methanol and DMS based methylotrophy and sulfur oxidation

A summary of the genetic potential of *Methylophaga thiooxydans* with particular relevance to methanol and DMS metabolism is given below.

Methanol oxidation:

The draft genome sequence of *M. thiooxydans* contains four genes encoding methanol dehydrogenases (MDH) including a Calcium-dependent methanol dehydrogenase (MxaFI, locus tag MDMS009_1502 encoding the alpha subunit, and MDMS009_1410, beta subunit) and three XoxF-type methanol dehydrogenases (MDMS009_1767, _2058 and _2642) which are homologs of the MDH enzymes shown to require rare earth elements as co-factors (Kjeltens et al. 2014). Genes *pqqBCDE* (encoded by MDMS009_2015, MDMS009_1956, MDMS009_1782, MDMS009_1752, respectively) involved in the synthesis of pyrroloquinoline quinone, the co-factor of MDH, are co-located in a four gene cluster in the vicinity of two of the XoxF encoding genes (MDMS009_1767 and MDMS009_2058).

Formaldehyde metabolism:

Formaldehyde generated through primary metabolism of C_1 substrates such as methanol, DMS and methanethiol can be conjugated to tetrahydromethanopterin (H_4MPT) by formaldehyde activating enzyme (Fae) for which two coding sequences are found (MDMS009_73 and MDMS009_595). There is a complete H_4MPT pathway for formaldehyde oxidation, comprising methylene-H_4MPT dehydrogenase (*mtdB*, MDMS009_1418), methenyl-H_4MPT-cyclohydrolase (*mch*, MDMS009_650), formylmethanofuran-H_4MPT-N-formyltransferase (*fhcD*, MDMS009_1442) and formyltransferase/hydrolase complex (*fhcA/fhcB/fhcC*, MDMS009_1429/ MDMS009_1334/ MDMS009_1520), which releases formate. Similarly, formate can be produced from C_1 groups fed into a tetrahydrofolate (H_4F) linked C_1-oxidation pathway comprising methylene-H_4F reductase (*metF*, MDMS009_515), a bifunctional methylene-H_4F-dehydrogenase/cyclohydrolase (*folD*, MDMS009_2980) which leads to production of formyl-H_4F which can then be degraded to formate either by formyl-H_4F-deformylase (*purU*, MDMS009_888) or the formate-H_4F ligase (*fhs*, MDMS009_1028) which couples the oxidation of formyl-H_4F to the production of ATP. The latter can also feed formate into the H_4F pathway to reduce formate for assimilatory purposes.

Finally, subunits of a formate dehydrogenase which can oxidise formate to CO_2 are encoded by MDMS009_2131, MDMS009_2239, MDMS009_2257 and MDMS009_1738.

M. thiooxydans has a ribulose monophosphate cycle (Entner–Doudoroff variant) for assimilation of formaldehyde including the enzymes 3-hexulose-6-phosphate synthase (*hxlA*, MDMS009_509), 6-phospho-3-hexuloisomerase (*hxlB*, MDMS009_475), fructose-bisphosphate aldolase (*fba*, MDMS009_1331), fructose-bisphosphatase (*fbp*_1, MDMS009_1532) and a transketolase (*tkt*, MDMS009_866).

Methanethiol and sulfide oxidation

Methylophaga has two genes encoding methanethiol oxidase (MDMS009_211 and MDMS009_768) contained in two distinct genomic regions which also encode a number of other genes involved in sulfur metabolism referred to hereafter as mto-cluster 1 and 2, respectively (Supplementary Figure S2). The gene order in both clusters is identical, with the major differences being that the mto-cluster1 contains two additional genes at the end. Functions encoded in both clusters include (i) the methanethiol oxidase, (ii) a fusion of SCO1 with MauG-type cytochrome *c* peroxidase, (iii) a SCO1/SenC/PrrC domain protein predicted to be involved in cytochrome maturation (identified by conserved domain search as a TlpA-like_family putative metallochaperone), (iv) homologs of persulfide dioxygenase (PDO) annotated as hydroxyacylglutathione hydrolases, (v) FAD-dependendent pyridine nucleotide-disulfide oxidoreductases (Sulfide quinone reductase B, SqrB), and (vi) a *sulP* gene encoding a sulfate/sulfite permease. In addition, mto-cluster 1 contains an adenylylsulfate kinase and a gene annotated as a 'methylated-DNA-protein-cysteine methyltransferase' in the same orientation, however to which extent the genes in the mto-clusters are co-regulated from one or more promoters is unclear.

In mto-cluster 2, the predicted methanethiol oxidase encoded by MDMS009_768 (465 amino acids [aa]) has a predicted signal peptide, but the mto-cluster 1 ortholog MDMS009_211 (427 aa) does not, prompting the question whether the former is periplasmic and the latter cytoplasmic. The region upstream of the CDS of MDMS009_211 has two alternative start codons, one of which would extend the encoded enzyme by 40 amino acids and result in a pre-MTO with a predicted signal peptide (SignalP 5.0 likelihood 98.2%, compared to 0.2% with previous start site, Supplementary Figure S3) as well as a more A/G rich ribosome binding site. In addition, for both mto-clusters the downstream *sco1/mauG* genes encoding proteins thought to be involved in MTO maturation are predicted to have a signal peptide. Together, these observations suggest that both MTO are periplasmic and are initially pre-proteins that are exported by the Sec pathway, where they are subsequently processed by the SCO1/MauG fusion proteins. The genes MDMS009_140 and MDMS009_894, immediately downstream of the SCO1/MauG genes, encoding SCO1/SenC/PrrC domain proteins are also expected to be periplasmic if an alternative start site of MDMS009_894 is taken into account (Supplementary Figure S4), which would result in products of similar length for these two proteins (168 and 164 aa, respectively), both with strong likelihood of containing a signal peptide (>98% likelihood).

Sulfide produced from MT oxidation can be oxidised by a flavocytochrome *c* sulfide dehydrogenase (*fccB*, MDMS009_2035; *fccA* MDMS009_753); genes encoding sulfide-quinone oxidoreductases include an *sqrA* MDMS009_1966 (based on homology to *Aquifex aeolicus sqrA)* as well as two *sqrB* (MDMS009_274, MDMS009_886). The location of these SQRs may be cytoplasmic or periplasmic, the confidence values for predicted signal peptide sequences are variable, 12% for MDMS009_274, 27% for MDMS009_1966, 55% for MDMS009_886. Candidate genes encoding persulfide dioxygenases include MDMS009_284 and MDMS009_765, both without signal peptides, suggesting these to be located in the cytoplasm. Permeases for sulfate or sulfite compounds are encoded by MDMS009_261 and MDMS009_921.

The genome of *M. thiooxydans* encodes some functions of the thiosulfate oxidation pathway including *soxCDHYZ*, as well as two genes encoding soxYZ fusions (MDMS009_1377 and 1373) but genes encoding SoxAB are missing suggesting this pathway cannot be used for oxidation of thiosulfate. Previous work suggested oxidation of thiosulfate to tetrathionate to occur, but the genome does not contain genes encoding any of the previously characterised thiosulfate to tetrathionate oxidising enzymes, including an *Allochromatium vinosum* type TsdA thiosulfate dehydrogenase (Denkmann et al., 2012), a *Shewanellea oneidensis* type octaheme tetrathionate reductase of (Atkinson et al., 2007) or a homolog of DoxDA as shown in *Acidianus* or *Acidithiobacillus* species (Wang et al., 2016).

The differential proteome of *Methylophaga thiooxydans* DMS010 from DMS and methanol grown cultures

Soluble and membrane protein fractions of *Methylophaga thiooxydans* DMS010 prepared for the proteomics analysis had concentrations ranging from 0.22 mg ml^{-1} to >1 mg ml^{-1} (Supplementary Table S3). All six soluble protein fractions were submitted for proteomic analysis. Identified peptides and proteins (minimum of 99% protein identity) were searched and placed into a pathway map using KEGG, Swiss-Prot, ExPASy and UniProt. A proposed pathway map of *Methylophaga thiooxydans* DMS010 grown with DMS is presented in Figure 1. A list of the corresponding proteins, genes, locus tag, and ratios of expression levels of the proteins expressed during growth on DMS or methanol can be found in Supplementary Table S4. Additional hypothetical proteins can be found in Supplementary Table S5.

Overall, up to 662 proteins (aggregate across triplicate samples) were identified in *Methylophaga thiooxydans* DMS010 when grown on methanol, from which up to 80 proteins

were up-regulated (at least 2-fold) compared to *Methylophaga thiooxydans* DMS010 growing on DMS as a carbon source. Up to 813 proteins were identified in *Methylophaga thiooxydans* DMS010 grown on DMS, of which up to 177 were up-regulated compared to growth on methanol. Proteins highly up-regulated in *Methylophaga thiooxydans* DMS010 growing in the presence of DMS were the methanethiol oxidases, of which *M. thiooxydans* encodes two (on average 7-fold and 18.67-fold, respectively, higher compared to methanol grown *M. thiooxydans* DMS010; Supplementary Table S4) in line with previous reports of methanethiol being a metabolic intermediate of its DMS metabolism (Schäfer, 2007;Boden et al., 2010).

Degradation of methanethiol via methanethiol oxidase (Mto – Figure 1) results in formation of formaldehyde, hydrogen sulfide (H_2S) and hydrogen peroxide (H_2O_2). H_2O_2 is toxic (Clifford and Repine, 1982;Thomas et al., 1994) and the catalase that decomposes it into water and oxygen was 14.67-fold up-regulated during growth on DMS (Figure 1). Similarly, peroxiredoxin was also up-regulated (3.75-fold) during growth on DMS, likely contributing to degradation of hydrogen peroxide or organic peroxides produced by H_2O_2 stress in the cell (Rhee, 2016).

The formaldehyde produced by methanethiol oxidase can either be degraded to CO_2 via formate or be assimilated into biomass via the RuMP cycle. Several enyzmes of the RuMP cycle were detected, such as 3-hexulose-6-phosphate synthase (MDMS009_509), 6-phospho-3-hexuloisomerase (MDMS009_475), transketolase (MDMS009_866), fructose-bisphosphate aldolase (MDMS009_1331) and fructose-bisphosphatase (MDMS009_1532). Formaldehyde degradation to formate and CO_2 appeared to occur via both the tetrahydromethanopterin-linked pathway and the tetrahydrofolate-linked pathway (Supplementary Table S4). The expression of most enzymes of these pathways showed only minor variation in expression levels between growth on methanol and DMS, although methylenetetrahydrofolate dehydrogenase (MtdA) was 5.30-fold up-regulated on DMS potentially indicating that one of the methyl groups of DMS may be oxidised via this pathway after its transfer onto tetrahydrofolate (H_4F) (Figure 1). In addition, the formate—tetrahydrofolate ligase (2.20-fold up-regulated) and the formyltetrahydrofolate deformylase of the tetrahydrofolate pathway were detected (Figure 1 and Supplementary Table S4). The expression of formate dehydrogenase was also shown (2.17-fold upregulated), which degrades the end-product of the tetrahydromethanopterin and tetrahydrofolate pathways, formate, to CO_2 (Figure 1). In addition, enzymes related to the glycolysis pathway such as the Glucose 6-phosphate isomerase (1.66-fold) that catalyses the conversion of D-glucose 6-phosphate to D-fructose 6-phosphate, the Phosphopyruvate hydratase (3.44-fold) or enzymes involved in the conversion of Glyceraldehyde-3-phosphate to Pyruvate were found upregulated. The enzymes dihydrolipoamide dehydrogenase (7.67-fold)

and dihydrolipoamide S-acetyltransferase (7.50-fold, E2 component of the pyruvate dehydrogenase) were also highly upregulated. Furthermore, proteins involved in the metabolism of amino and nucleotide sugars, and lipopolysaccharide biosynthesis were also detected. The oxidation of the sulfur of DMS is of major importance as an intermediate in the sulfur cycle. The initial step of DMS degradation and the subsequent oxidation of the sulfur are summarized in Figures 1 and 2. An enzyme of sulfide oxidation detected in the proteome of DMS-grown cells included sulfide dehydrogenase (MDMS009_2035; encoding FccB), which was upregulated 1.67-fold.

The differential transcriptome of *Methylophaga thiooxydans* DMS010 from DMS and methanol grown cultures

RNA extraction and rRNA depletion led to successful mRNA enrichment. Results of the RNA analysis using the Bioanalyzer can be found in Supplementary Figure S5. Concentrations of mRNA preparations from *M. thiooxydans* grown on methanol ranged from 14.5 to 26.98 pg/µL and for *M. thiooxydans* grown on DMS from 85.7 to 172.0 pg/µL. These mRNA preparations were used for sequencing library preparation and high-throughput sequencing of cDNA (from mRNA). Overall, between 82 and 89% of the transcriptomic reads of the triplicate DMS and methanol treatments were successfully aligned to the *Methylophaga thiooxydans* DMS010 genome. Between 23 and 27% aligned to unannotated regions. 1228 5'UTRs and 1309 3'UTRs (untranslated regions) were detected and 176,974 RNAs were predicted. From these RNA transcripts an overall 3,078 RNAs coding for proteins were predicted. Of those protein-coding transcripts, 280 were highly up-regulated (2.5-fold to 469-fold) and another 1,340 protein-coding transcripts were up-regulated at least 2-fold during growth on DMS compared to methanol. When *Methylophaga thiooxydans* was grown on methanol, 230 protein-coding transcripts were highly up-regulated (2.5-fold to 52-fold). Transcripts coding for 936 hypothetical proteins were detected, of which 133 were highly up-regulated (at least 2.5-fold) during growth on DMS. In total, 115 operons were detected by Rockhopper during the transcriptome analysis. Supplementary Table S6 summarizes the main results of the transcriptomic experiment by indicating the protein-encoding genes with locus tags and their expression level in *Methylophaga thiooxydans* when grown either with methanol or DMS. Highly up-regulated transcripts in the DMS treatment compared to methanol treatment were those of the genes encoding for methanethiol oxidase (MDMS009_211, MDMS009_768) and catalase/peroxidase (MDMS009_1525, MDMS009_2469), dihydrolipoamide dehydrogenase (MDMS009_809, a component of the pyruvate, α-ketoglutarate, and branched-chain amino acid-dehydrogenase complexes and the glycine cleavage system), several hypothetical proteins,

proteins involved in coenzyme PQQ synthesis (*pqqE*, *pqqD* and *pqqB*), a membrane-bound protein (MDMS009_906) potentially involved in long chain fatty acid transport (porin), a fatty acid desaturase (MDMS009_226) and several others (Supplementary Table S6). Highly up-regulated transcripts in the methanol treatment compared to the DMS treatment included proteins involved in siderophore (high-affinity iron-chelating compounds) biosynthesis and methanol dehydrogenase proteins (Supplementary Table S6).

Comparison of proteomics and transcriptomics data

A comparison of proteomics and transcriptomics data based on mapping the expression data of both approaches onto the *M. thiooxydans* genome using the CGView comparison tool in-house pipeline (Grant et al., 2012) is shown in Supplementary Figure S6. Overall, there was good agreement between the two approaches, whereby the transcriptome data showed additional detail, which is not surprising due to high-throughput sequencing providing more in-depth analysis and higher coverage (Hegde et al., 2003;Trapp et al., 2017) and the omission of the membrane fraction for proteomics analysis, for instance. The analysis against the genome assembly identified the activation of specific regions of the genome strongly supporting coordinate gene expression depending on the growth substrate (Supplementary Figure S6).

Comparative genome analysis of *Methylophaga* isolates

A comparative genome analysis was carried out to assess to what extent genes of DMS metabolism detected in *M. thiooxydans* are conserved in other members of the genus *Methylophaga*, which differ in their reported abilities to degrade methylated sulfur compounds. At the time of the analysis, genomes for six *Methylophaga* genomes obtained from different environments were chosen (Supplementary Table S7), including three for which growth on DMS has been reported previously (*M. thiooxydans*, *M. sulfidovorans*, *M. aminisulfidivorans*), one which was shown not to grow on DMS (*M. lonarensis*) and two for which DMS degradation has not been tested (*M. frappieri* and *M. nitratireducenticrescens* strain JAM1). Genome sizes range from ~2.64 Mb to ~3.26 Mb with GC contents between 40% to 50% (Supplementary Table S1). Analysis of sequence annotations revealed that on average 91% of the genomes consist of coding sequences. Pan-genome analysis, carried out using EDGAR (Blom et al., 2016), identified metabolic genes present in all *Methylophaga* species (core genes), in two or more *Methylophaga* species (accessory or dispensable genes), and 'unique' *Methylophaga* species (singleton genes). A pan-genome tree was constructed (Figure 3A) based on the pan-

genome dataset and neighbor-joining method (Saitou and Nei, 1987). The *Methylophaga* species exhibiting the least amount of evolutionary change from a common ancestor are *M. aminisulfidivorans* and *M. sulfidovorans* (Figure 3A), these two species also have a higher average amino acid identity score compared to the other four *Methylophaga* species (Supplementary Figure S7). Overall, pan-genome analysis of the six strains identified a total of 11,316 genes, consisting of 1,397 core genes, 6105 dispensable genes and 323, 651, 396, 323, 513 and 211 singletons for *M. thiooxydans*, *M. frappieri* strain JAM7, *M. aminisulfidivorans*, *M. lonarensis, M. nitratireducenticrescens* and *M. sulfidovorans*, respectively (Figure 3B). On average 56.1% of singletons were identified as having hypothetical functions (Figure 3C). The number of singletons did not correlate with the size of the genome, which contrasts with the correlation of the number of genes and the size of the genome.

Investigation of DMS utilization pathways and subsequent C1 oxidation pathways in six *Methylophaga* species revealed the presence of the gene encoding for the methanethiol oxidase involved in degradation of the metabolic intermediate MT in the genomes of *M. thiooxydans* and *M. sulfidovorans* while this gene was not detected in *M. frappieri* strain JAM7, *M. aminisulfidivorans*, *M. lonarensis* and *M. nitratireducenticrescens* (Supplementary Table S8). None of these *Methylophaga* species contain other known genes of DMS metabolism, including homologues of the DMS monooxygenase (*dmoA*) (Boden et al., 2011a) or the DMS dehydrogenase (McDevitt et al., 2002).

Regarding methanol metabolism, all six *Methylophaga* genomes have genes encoding the lanthanide-dependent methanol dehydrogenase XoxF (Hibi et al., 2011;Nakagawa et al., 2012;Pol et al., 2014) of the clade XoxF5 (Keltjens et al., 2014) and the *mxaF* gene, encoding the alpha subunit of the Calcium-dependent methanol dehydrogenase (Williams et al., 2005), which are responsible for the oxidation of methanol to formaldehyde (Harms et al., 1996;Chistoserdova and Lidstrom, 1997) (Figure 4). The lanthanide-dependent methanol dehydrogenase is thought to be more widespread in bacterial genomes (Lv et al., 2018).

All six *Methylophaga* species have genes encoding enzymes of the tetrahydrofolate (H_4F) linked pathway (Figure 4). Genes encoding 5,10-methylenetetrahydrofolate reductase (*metF*) and the bifunctional enzyme 5,10-methylene-tetrahydrofolate dehydrogenase/cyclohydrolase (*folD*), were detected in all the *Methylophaga* genomes (Figure 4). The formate-tetrahydrofolate ligase, encoded by the gene *fhs* (Figure 4), can have a role in catabolic and

anabolic metabolism, either allowing for ATP synthesis during oxidation of formyl-H4F (CHO-H_4F) to formate or providing C1 units for assimilatory metabolism through energy dependent conversion of formate to formyl tetrahydrofolate; alternatively the oxidation of formyl-H_4F to formate can also be facilitated by PurU, the formyl-H_4F deformylase encoded by the *purU* gene (Marx et al 2003, Studer et al. 2003, Chen, 2012).

Similarly, genes encoding the formaldehyde activating enzyme (*fae*) and further enzymes of the tetrahydromethanopterin (H_4MPT)-linked pathway of formaldehyde oxidation are also present in all *Methylophaga* genomes. The formate dehydrogenase (FDH, encoded by gene *fdh*) mediates the last step of the H_4MPT and H_4F-linked C_1 oxidation pathways, the oxidation of formate to CO_2. The genes encoding FDH are present in all *Methylophaga* genomes. Reduction of formaldehyde can also be mediated by the glutathione-dependent formaldehyde-activating enzyme, encoded by the gene *gfa* (Goenrich et al., 2002), which was detected in all six *Methylophaga* species, however, the gene encoding for the glutathione-dependent formaldehyde dehydrogenase (*adhI*) was only detected in *M. aminisulfidivorans* and *M. lonarensis* and the gene encoding the S-formyl-GSH hydrolase (*fghA*) was not detected in any of these *Methylophaga* species (Figure 4).

Thus, as expected, comparative genome analysis shows that all six *Methylophaga* species are capable of generating energy from methanol using either lanthanide-dependent or the calcium-dependent methanol dehydrogenases and have pathways of H_4F and H_4MPT-linked formaldehyde oxidation. Regarding sulfur metabolism, two *Methylophaga* isolates *M. thiooxydans* and *M. sulfidovorans* may have a common DMS oxidation pathway involving an as-yet unidentified primary enzyme of DMS metabolism suggested to be a DMS methyltransferase (Boden et al., 2010) and methanethiol oxidase, with subsequent formaldehyde oxidation via the H_4F-dependent pathway and/or the H_4MPT-dependent pathways. *Methylophaga aminisulfidivorans,* which was reported to grow on DMS and DMSO (Kim et al., 2007), does not have a methanethiol oxidase (*mtoX*). This strain must either possess an as-yet unidentified pathway for the degradation of DMS (and DMSO) or use an alternative methanethiol oxidase. For the oxidation of potentially DMS or DMSO-derived sulfur (sulfide) *Methylophaga aminisulfidivorans* has a sulfide dehydrogenase (*fccAB*) (Figure 4). Regarding inorganic sulfur metabolism, homologs of genes encoding enzymes of sulfide oxidation were found in all strains, but neither SQR nor sulfide dehydrogenases are present in all strains (Figure 4). All strains have genes for assimilation of sulfate to sulfite, and, with the exception of *M.*

frappieri all strains had a sulfite reductase. None of the strains contain a complete *sox* system, but some components encoded by the genes *soxCDYZH* were present in most genomes.

Discussion

Overall, comparative proteomic and transcriptomic experiments of *Methylophaga thiooxydans* grown on either DMS or methanol identified the major pathways involved in *Methylophaga thiooxydans* during growth on DMS and methanol.

The comparative proteomics experiment of *Methylophaga thiooxydans* grown either on DMS or methanol showed a great coverage of proteins across several relevant metabolic pathways, including the primary enzymes involved in methanol degradation, methanethiol degradation, functions involved in formaldehyde degradation and assimilation (such as the KEGG category one-carbon pool by folate, the RuMP cycle, KEGG category methane metabolism) as well as relevant enzymes from central carbon metabolism. Similarly, the transcriptomics analysis identified transcripts of relevant genes, but additional genes activated in either growth condition were also identified.

Pathways induced during growth on DMS

Both methanethiol oxidases present in the genome of *M. thiooxydans* were highly upregulated during growth on DMS as shown by both proteomics and transcriptomics. The downstream genes encoding a fusion of the SCO1/MauG domains present as single genes in *Hyphomicrobium* sp VS, which are suggested to be important in maturation of the protein, were also both induced during growth on DMS. In line with MT degradation producing H_2O_2, induction of catalase and peroxiredoxin reflected important responses to oxidative stress (Rhee, 2016). Membrane lipids are likely also damaged by H_2O_2 which may explain the upregulation of genes that might contribute to maintenance of membrane lipid homeostasis including for instance a fatty acid desaturase (MDMS009_226) which may potentially receive reducing power from the product of MDMS009_61, an NAD(P)H flavin reductase that was highly upregulated during growth on DMS (Supplementary Table S6).

What is missing, as far as primary DMS metabolism is concerned, is the presumed DMS methyltransferase which was suggested to be responsible for the first step of DMS degradation in *M. thiooxydans* (Boden et al., 2010). A candidate gene for this putative 'DMS-methyltransferase' had not been identified so far and none was detected here as being induced

on DMS by proteomics or transcriptomics. There is a methyltransferase in mto-cluster 1, annotated as a 'methylated DNA-protein cysteine S-methyltransferase'. Although its transcription can be seen in the RNAseq data, the expression levels are similar during growth on methanol and DMS and the protein is not detected in the proteomics data. It most likely plays a role in DNA repair.

Proteomics data indicated that the enzymes of the H_4F-linked oxidation pathway were upregulated. Thus a methyl group transfer from DMS to H_4F with its subsequent H_4F-linked oxidation to CO_2 is a possibility, similar to the oxidation of the methyl group from methyl chloride in a H_4F-linked degradation pathway first described in *Methylobacterium extorquens* CM4 (Vannelli et al., 1999). Cleaving off one methyl group from DMS could result in methanethiol as a reaction product, consistent with observed MT oxidation by the presence and expression of MTO (Schäfer, 2007;Boden et al., 2010). The expression of the H_4F pathway on DMS and methanol suggests that it does not solely serve as a catabolic route though. An alternative, unrecognised route of primary DMS metabolism may exist, such as a hydrolase or monooxygenase. This issue warrants further work, potentially using ^{13}C labelling and metabolomic analysis. Formaldehyde is also channelled into the tetrahydromethanopterin-linked pathway of formaldehyde degradation by formaldehyde activating enzyme and degraded to CO_2.

Proteomics and transcriptomics data support that formaldehyde produced by MTO is assimilated into biomass via the RuMP cycle. Relevant enzymes were detected by both the proteomics and transcriptomics analyses. However, Boden et al. (2010) previously detected activity of the 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase (locus MDMS009_149) but none of fructose-1,6-bisphophate aldolase. Previous analysis of the draft genome sequence of *Methylophaga thiooxydans* suggested that a fructose-1,6-bisphophate aldolase was absent (Boden et al., 2011b), but a reanalysis showed that the protein-encoding gene for the fructose-1,6-bisphophate aldolase was present in the *Methylophaga thiooxydans* genome (MDMS009_1331). Proteomics and transcriptomics analysis showed that fructose-1,6-bisphophate aldolase was expressed, suggesting that enzyme activities of the RuMP cycle need to be reassessed in *Methylophaga thiooxydans*.

Sulfide oxidation

Previous work suggested that sulfide dehydrogenase and sulfide oxygenase activities were present. A sulfide dehydrogenase (flavocytochrome *c* sulfide dehydrogenase) was induced

during growth on DMS, which produces elemental sulfur. Transcriptomics demonstrated that a second sulfide-degrading enzyme, the sulfide:quinone reductase (SQR) was also highly expressed in cells grown on DMS (locus MDMS009_1966) but not on methanol, which would produce polysulfide, which could then react through an unknown enzyme or chemically with sulfite to yield thiosulfate ($S_2O_3^{2-}$, Figure 2) as previously found in *Thiobacillus thioparus* and *Acidithiobacillus thiooxidans* (Suzuki and Silver, 1966) and suggested for *Methylophaga thiooxydans* (Boden et al., 2010). The sulfite for this reaction was suggested to be the product of the sulfide oxygenase whose activity was previously measured in *Methylophaga thiooxydans*, with the most likely candidates for this reaction being the induced persulfide oxygenases encoded in the vicinity of MTO genes. Several enzymes/genes could therefore be demonstrated which are in agreement with the previous work on the sulfur oxidation pathway in *Methylophaga* (Boden et al., 2010). The final step of this pathway, the oxidation of thiosulfate to tetrathionate has previously been shown to be carried out in *Allochroatium vinosum* by the TsdA thiosulfate dehydrogenase (Denkmann et al., 2012), in *Shewanellea oneidensis* by the octaheme tetrathionate reductase (Atkinson et al., 2007) or DoxDA *Acidianus* or *Acidithiobacillus* (Wang et al., 2016), but *M. thiooxydans* does not have homologs of these enzymes. A recent study by Pyne and colleagues (Pyne et al., 2018) demonstrated that XoxF PQQ-dependendent dehydrogenases in *Advenella kashmirensis* were capable of oxidising thiosulfate to tetrathionate. Given the expression of several XoxF enzymes in *Methylophaga thiooxydans* both during growth on DMS and methanol, it is possible that the XoxF enzymes may be responsible for the this step of the sulfur oxidation pathway, but this requires experimental confirmation of this activity for any of these enzymes which were previously assumed to have a role in methanol degradation. The expression of some Sox proteins at higher abundance during growth on DMS suggests that these may play a role in oxidation of inorganic sulfur intermediates. Genes encoding SoxA and SoxB were missing, in agreement with the observation that thiosulfate is not oxidised to sulfate in *M. thiooxydans*.

Differences in gene expression during growth on methanol

Highly overexpressed genes in *Methylophaga thiooxydans* grown on methanol were those for the Calcium-dependent methanol dehydrogenase and for proteins involved in anthrachelin biosynthesis. Upregulation of the methanol dehydrogenase subunits and the PQQ biosynthesis genes that were induced would be expected. Anthrachelin is a siderophore involved in iron acquisition (Neilands, 1995;Garner et al., 2004). The methanol dehydrogenase is a PQQ-dependent methanol dehydrogenase, and the pyrrolo quinoline quinone (PQQ) accepts the

electrons produced during methanol oxidation and passes them to cytochrome *c*, which is an iron containing haemprotein, which might explain why protein-encoding genes involved in the biosynthesis of the iron acquiring siderophore anthrachelin are up-regulated during growth on methanol.

Hypothetical genes

A number of previously unannotated and/or not even predicted genes (Supplementary Table S9) were identified as being expressed. A total of 936 protein-coding transcripts were detected for which no function could be predicted, which accounts for about 30% of all detected protein-coding transcripts. About 20 to 40% of hypothetical proteins are normal in a sequenced genome (Galperin, 2001). For some genes annotated as hypothetical proteins their expression during growth on DMS or methanol associates them with as-yet uncharacterised roles during growth on specific carbon sources and so a first potential association with a metabolic process has been achieved. Further work on these genes will be critical to better understand the specific roles these genes play for growth on DMS or methanol by *Methylophaga*. It reemphasises the need to establish a genetic system for studying gene function in *Methylophaga thiooxydans*. Some transcripts were identified as previously unannotated genes. None of these had a predicted function (Supplementary Table S9), but some were highly expressed.

Based on molecular genetics a strong correlation between mRNA expression levels and protein abundance would be assumed (Zhang et al., 2010). However, several studies have failed to demonstrate a high correlation between protein and mRNA abundances (Greenbaum et al., 2002;Nie et al., 2007;Taniguchi et al., 2010;Vogel and Marcotte, 2012). This contradiction underlines the benefit of using proteomics and transcriptomics in combination as application of just one of these approaches is likely to be less representative of the biological system under investigation (Park et al., 2005;Zhang et al., 2010). How well proteomics and transcriptomics results matched one another is shown in Supplementary Figure S6, which included all transcriptomic and proteomic expression levels. In this study, some proteins were found in higher abundance in *Methylophaga thiooxydans* grown on DMS and their corresponding protein encoding genes were often also up-regulated in the transcriptomic experiment (Supplementary Figure S6), e.g. the methanethiol oxidase (MtoX). However, that was only the case for a few proteins. There were also cases where results from proteomic and transcriptomics

were somewhat contradictory, e.g. the observation of higher protein levels of a sulfate permease on DMS and higher transcript levels of the same protein on methanol.

Conclusions

A combined approach of proteomics and transcriptomics analysis has provided more detailed information on pathways involved in DMS and methanol degradation in *M. thiooxydans*, confirming some previous observations made by enzyme assays, and in some cases identifying the genes and proteins responsible for specific activities. At the same time, the data also identify gaps of understanding and identify specific issues that require further investigation, such as the primary mechanism of DMS degradation and the potential role in thiosulfate oxidation of the lanthanide-dependent XoxF methanol dehydrogenases during growth on DMS. Pangenomic analysis showed, as expected for a genus of methylotrophic bacteria, that relevant central pathways such as H_4F and H_4MPT-linked formaldehyde and methanol degradation are part of the core genome, but only two of the six investigated *Methylophaga* genomes (*M. thiooxydans* and *M. sulfidovorans*) indicated metabolic potential to utilize methanethiol, the intermediate of DMS degradation, based on the previously identified methanethiol oxidase. This is surprising given the reported ability of *M. aminisulfidivorans* to grow on DMSO and DMS, as well as the close relatedness between *M. sulfidovorans* and *M. aminisulfidivorans* based on the relatively smallest evolutionary change. Overall, these results demonstrate that our understanding of the underlying traits for utilisation of methylated sulfur compounds in members of this genus are still inadequate and that more work is required to characterise these metabolic functions using a range of genetics, biochemistry and metabolomics approaches.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We thank Nataliia Myronova for helping with preparation of protein fractions for proteomics analysis, the Proteomics Research Technology Platform at the University of Warwick, especially Susan Slade for mass spectrometry analysis of protein extracts and Andrew Bottrill for help with data submission to PRIDE. We thank Jeanette Selby for technical support in the School of Life Sciences Genomics facility; Paul Stothard for using their in-house pipeline of the CGView comparison tool. The EDGAR platform is financially supported by the BMBF

grant FKZ 031A533 within the de.NBI network. Work in HS laboratory was supported by NERC grant NE/E013333/1. Eileen Kröber gratefully acknowledges support of her PhD studies through support by the DAAD, the Richard-Winter Stiftung, and NERC. We are grateful to Richard Fetherston for feedback on the manuscript and Christiane Dahl for helpful discussions on inorganic sulfur metabolism.

REFERENCES

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology* 215**,** 403-410.

Armenteros, J.J.A., Tsirigos, K.D., Sønderby, C.K., Petersen, T.N., Winther, O., Brunak, S., Von Heijne, G., and Nielsen, H.J.N.B. (2019). SignalP 5.0 improves signal peptide predictions using deep neural networks. 1.

Atkinson, S.J., Mowat, C.G., Reid, G.A., and Chapman, S.K.J.F.L. (2007). An octaheme c‐type cytochrome from Shewanella oneidensis can reduce nitrite and hydroxylamine. 581**,** 3805-3808.

Aziz, R.K., Bartels, D., Best, A.A., Dejongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Glass, E.M., and Kubal, M. (2008). The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 9**,** 75.

Blom, J., Kreis, J., Spanig, S., Juhre, T., Bertelli, C., Ernst, C., and Goesmann, A. (2016). EDGAR 2.0: an enhanced software platform for comparative gene content analyses. *Nucleic Acids Research* 44**,** W22-W28.

Boden, R., Borodina, E., Wood, A.P., Kelly, D.P., Murrell, J.C., and Schäfer, H. (2011a). Purification and characterization of dimethylsulfide monooxygenase from *Hyphomicrobium sulfonivorans*. *Journal of Bacteriology* 193**,** 1250-1258.

Boden, R., Ferriera, S., Johnson, J., Kelly, D.P., Murrell, J.C., and Schäfer, H. (2011b). Draft genome sequence of the chemolithoheterotrophic, halophilic methylotroph *Methylophaga thiooxydans* DMS010. *Journal of Bacteriology* 193**,** 3154-3155.

Boden, R., Kelly, D.P., Murrell, J.C., and Schäfer, H. (2010). Oxidation of dimethylsulfide to tetrathionate by *Methylophaga thiooxidans* sp. nov.: a new link in the sulfur cycle. *Environmental Microbiology* 12**,** 2688-2699.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72**,** 248-254.

Carslaw, K.S., Boucher, O., Spracklen, D.V., Mann, G.W., Rae, J.G.L., Woodward, S., and Kulmala, M. (2010). A review of natural aerosol interactions and feedbacks within the Earth system. *Atmospheric Chemistry and Physics* 10**,** 1701-1737.

Charlson, R.J., Lovelock, J.E., Andreae, M.O., and Warren, S.G. (1987). Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature* 326**,** 655-661.

Chistoserdova, L., and Lidstrom, M.E. (1997). Molecular and mutational analysis of a DNA region separating two methylotrophy gene clusters in *Methylobacterium extorquens* AM1. *Microbiology* 143**,** 1729-1736.

Clifford, D.P., and Repine, J.E. (1982). Hydrogen peroxide mediated killing of bacteria. *Molecular and Cellular Biochemistry* 49**,** 143-149.

Curson, A.R.J., Todd, J.D., Sullivan, M.J., and Johnston, A.W.B. (2011). Catabolism of dimethylsulphoniopropionate: microorganisms, enzymes and genes. *Nature Reviews Microbiology* 9**,** 849-859.

Curson, A.R.J., Williams, B.T., Pinchbeck, B.J., Sims, L.P., Martinez, A.B., Rivera, P.P.L., Kumaresan, D., Mercade, E., Spurgin, L.G., Carrion, O., Moxon, S., Cattolico, R.A., Kuzhiumparambil, U., Guagliardo, P., Clode, P.L., Raina, J.B., and Todd, J.D. (2018). DSYB catalyses the key step of dimethylsulfoniopropionate biosynthesis in many phytoplankton. *Nature Microbiology* 3**,** 430-439.

De Bont, J.a.M., Van Dijken, J.P., and Harder, W. (1981). Dimethyl sulphoxide and dimethyl sulphide as a carbon, sulphur and energy source for growth of *Hyphomicrobium* S. *Microbiology* 127**,** 315-323.

De Zwart, J., Sluis, J., and Kuenen, J.G. (1997). Competition for dimethyl sulfide and hydrogen sulfide by *Methylophaga sulfidovorans* and *Thiobacillus thioparus* T5 in continuous cultures. *Applied and Environmental Microbiology* 63**,** 3318-3322.

Denkmann, K., Grein, F., Zigann, R., Siemen, A., Bergmann, J., Van Helmont, S., Nicolai, A., Pereira, I.A., and Dahl, C. (2012). Thiosulfate dehydrogenase: a widespread unusual acidophilic *c*-type cytochrome. *Environmental Microbiology* 14**,** 2673-2688.

Galperin, M.Y. (2001). Conserved 'hypothetical' proteins: new hints and new puzzles. *Comparative and Functional Genomics* 2**,** 14-18.

Garner, B.L., Arceneaux, J.E., and Byers, B.R. (2004). Temperature control of a 3,4-dihydroxybenzoate (protocatechuate)-based siderophore in *Bacillus anthracis*. *Current Microbiology* 49**,** 89-94.

Goenrich, M., Bartoschek, S., Hagemeier, C.H., Griesinger, C., and Vorholt, J.A. (2002). A glutathione-dependent formaldehyde-activating enzyme (Gfa) from *Paracoccus denitrificans* detected and purified via two-dimensional proton exchange NMR spectroscopy. *Journal of Biological Chemistry* 277**,** 3069-3072.

Grant, J.R., Arantes, A.S., and Stothard, P. (2012). Comparing thousands of circular genomes using the CGView Comparison Tool. *BMC Genomics* 13**,** 1-8.

Greenbaum, D., Jansen, R., and Gerstein, M. (2002). Analysis of mRNA expression and protein abundance data: an approach for the comparison of the enrichment of features in the cellular population of proteins and transcripts. *Bioinformatics* 18**,** 585-596.

Harms, N., Ras, J., Koning, S., Reijnders, W.N.M., Stouthamer, A.H., and Spanning, R.G.M. (1996). "Genetics of C1 metabolism regulation in *Paracoccus denitrificans*," in *Microbial growth on C(1) compounds,* eds. M.E. Lidstrom & F.R. Tabita. (Dordrecht, the Netherlands: Kluwer Academic Publishers), 126-132.

Hegde, P.S., White, I.R., and Debouck, C. (2003). Interplay of transcriptomics and proteomics. *Current Opinion in Biotechnology* 14**,** 647-651.

Hibi, Y., Asai, K., Arafuka, H., Hamajima, M., Iwama, T., and Kawai, K. (2011). Molecular structure of La3+-induced methanol dehydrogenase-like protein in *Methylobacterium radiotolerans*. *Journal of Bioscience and Bioengineering* 111**,** 547-549.

Janvier, M., and Grimont, P.A. (1995). The genus *Methylophaga*, a new line of descent within phylogenetic branch gamma of Proteobacteria. *Research in Microbiology* 146**,** 543-550.

Keltjens, J.T., Pol, A., Reimann, J., and Op Den Camp, H.J.M. (2014). PQQ-dependent methanol dehydrogenases: rare-earth elements make a difference. *Applied Microbiology and Biotechnology* 98**,** 6163-6183.

Kiene, R.P., and Bates, T.S. (1990). Biological removal of dimethyl sulfide from sea-water. *Nature* 345**,** 702-705.

Kim, H.G., Doronina, N.V., Trotsenko, Y.A., and Kim, S.W. (2007). *Methylophaga aminisulfidivorans* sp. nov., a restricted facultatively methylotrophic marine bacterium. *International Journal of Systematic and Evolutionary Microbiology* 57**,** 2096-2101.

Lana, A., Bell, T.G., Simó, R., Vallina, S.M., Ballabrera-Poy, J., Kettle, A.J., Dachs, J., Bopp, L., Saltzman, E.S., Stefels, J., Johnson, J.E., and Liss, P.S. (2011). An updated climatology of surface dimethlysulfide concentrations and emission fluxes in the global ocean. *Global Biogeochemical Cycles* 25**,** n/a-n/a.

Lidbury, I., Kröber, E., Zhang, Z.D., Zhu, Y.J., Murrell, J.C., Chen, Y., and Schäfer, H. (2016a). A mechanism for bacterial transformation of dimethylsulfide to dimethylsulfoxide: a missing link in the marine organic sulfur cycle. *Environmental Microbiology* 18**,** 2754-2766.

Lidbury, I.D., Murphy, A.R., Scanlan, D.J., Bending, G.D., Jones, A.M., Moore, J.D., Goodall, A., Hammond, J.P., and Wellington, E.M. (2016b). Comparative genomic, proteomic and exoproteomic analyses of three *Pseudomonas* strains reveals novel insights into the phosphorus scavenging capabilities of soil bacteria. *Environmental Microbiology* 18**,** 3535-3549.

Lomans, B.P., Van Der Drift, C., Pol, A., and Op Den Camp, H.J.M. (2002). Microbial cycling of volatile organic sulfur compounds. *Cellular and Molecular Life Science* 59**,** 575-588.

Lovelock, J.E., Maggs, R.J., and Rasmussen, R.A. (1972). Atmospheric dimethyl sulphide and the natural sulphur cycle. *Nature* 237**,** 452-453.

Lv, H., Sahin, N., and Tani, A. (2018). Draft genome and description of *Novimethylophilus kurashikiensis* gen. nov. sp. nov., a new lanthanide-dependent methylotrophic species of *Methylophilaceae*. *Environmental Microbiology*.

Markowitz, V.M., Chen, I.-M.A., Palaniappan, K., Chu, K., Szeto, E., Pillay, M., Ratner, A., Huang, J., Woyke, T., and Huntemann, M. (2013). IMG 4 version of the integrated microbial genomes comparative analysis system. *Nucleic acids research* 42**,** D560-D567.

Mcclure, R., Balasubramanian, D., Sun, Y., Bobrovskyy, M., Sumby, P., Genco, C.A., Vanderpool, C.K., and Tjaden, B. (2013). Computational analysis of bacterial RNA-Seq data. *Nucleic Acids Research* 41.

Mcdevitt, C.A., Hugenholtz, P., Hanson, G.R., and Mcewan, A.G. (2002). Molecular analysis of dimethyl sulphide dehydrogenase from *Rhodovulum sulfidophilum*: its place in the dimethyl sulphoxide reductase family of microbial molybdopterin-containing enzymes. *Molecular Microbiology* 44**,** 1575-1587.

Nakagawa, T., Mitsui, R., Tani, A., Sasa, K., Tashiro, S., Iwama, T., Hayakawa, T., and Kawai, K. (2012). A Catalytic Role of XoxF1 as La^{3+}-Dependent Methanol Dehydrogenase in *Methylobacterium extorquens* Strain AM1. *Plos One* 7.

Neilands, J.B. (1995). Siderophores: structure and function of microbial iron transport compounds. *Journal of Biological Chemistry* 270**,** 26723-26726.

Nie, L., Wu, G., Culley, D.E., Scholten, J.C., and Zhang, W. (2007). Integrative analysis of transcriptomic and proteomic data: challenges, solutions and applications. *Critical Reviews in Biotechnology* 27**,** 63-75.

Park, S.J., Lee, S.Y., Cho, J., Kim, T.Y., Lee, J.W., Park, J.H., and Han, M.J. (2005). Global physiological understanding and metabolic engineering of microorganisms based on omics studies. *Applied Microbiology and Biotechnology* 68**,** 567-579.

Pol, A., Barends, T.R.M., Dietl, A., Khadem, A.F., Eygensteyn, J., Jetten, M.S.M., and Op Den Camp, H.J.M. (2014). Rare earth metals are essential for methanotrophic life in volcanic mudpots. *Environmental Microbiology* 16**,** 255-264.
Pyne, P., Alam, M., Rameez, M.J., Mandal, S., Sar, A., Mondal, N., Debnath, U., Mathew, B., Misra, A.K., and Mandal, A.K.J.M.M. (2018). Homologs from sulfur oxidation (Sox) and methanol dehydrogenation (Xox) enzyme systems collaborate to give rise to a novel pathway of chemolithotrophic tetrathionate oxidation. 109**,** 169-191.
Rhee, S.G. (2016). Overview on Peroxiredoxin. *Molecules and Cells* 39**,** 1-5.
Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution* 4**,** 406-425.
Schäfer, H. (2007). Isolation of *Methylophaga* spp. from marine dimethylsulfide-degrading enrichment cultures and identification of polypeptides induced during growth on dimethylsulfide. *Applied and Environmental Microbiology* 73**,** 2580-2591.
Schäfer, H., Myronova, N., and Boden, R. (2010). Microbial degradation of dimethylsulphide and related C1-sulphur compounds: organisms and pathways controlling fluxes of sulphur in the biosphere. *Journal of Experimental Botany* 61**,** 315-334.
Simó, R. (2001). Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links. *Trends in Ecology & Evolution* 16**,** 287-294.
Stiefel, E.I. (1996). Molybdenum bolsters the bioinorganic brigade. *Science* 272**,** 1599-1600.
Suzuki, I., and Silver, M. (1966). The initial product and properties of the sulfur-oxidizing enzyme of *Thiobacilli*. *Biochimica et Biophysica Acta* 122**,** 22-33.
Taniguchi, Y., Choi, P.J., Li, G.W., Chen, H., Babu, M., Hearn, J., Emili, A., and Xie, X.S. (2010). Quantifying *E. coli* proteome and transcriptome with single-molecule sensitivity in single cells. *Science* 329**,** 533-538.
Tatusov, R.L., Galperin, M.Y., Natale, D.A., and Koonin, E.V. (2000). The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Research* 28**,** 33-36.
Thomas, E.L., Milligan, T.W., Joyner, R.E., and Jefferson, M.M. (1994). Antibacterial activity of hydrogen peroxide and the lactoperoxidase-hydrogen peroxide-thiocyanate system against oral Streptococci. *Infection and Immunity* 62**,** 529-535.
Thompson, A.S., Owens, N.J.P., and Murrell, J.C. (1995). Isolation and characterization of methanesulfonic acid-degrading bacteria from the marine-environment. *Applied and Environmental Microbiology* 61**,** 2388-2393.
Trapp, J., Mcafee, A., and Foster, L.J. (2017). Genomics, transcriptomics and proteomics: enabling insights into social evolution and disease challenges for managed and wild bees. *Molecular Ecology* 26**,** 718-739.
Vannelli, T., Messmer, M., Studer, A., Vuilleumier, S., and Leisinger, T. (1999). A corrinoid-dependent catabolic pathway for growth of a *Methylobacterium* strain with chloromethane. *Proceedings of the National Academy of Sciences of the United States of America* 96**,** 4615-4620.
Visscher, P.T., and Taylor, B.F. (1993). A new mechanism for the aerobic catabolism of dimethyl sulfide. *Applied and Environmental Microbiology* 59**,** 3784-3789.
Vogel, C., and Marcotte, E.M. (2012). Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nature Reviews Genetics* 13**,** 227-232.

Wang, Z.-B., Li, Y.-Q., Lin, J.-Q., Pang, X., Liu, X.-M., Liu, B.-Q., Wang, R., Zhang, C.-J., Wu, Y., and Lin, J.-Q.J.F.I.M. (2016). The two-component system RsrS-RsrR regulates the tetrathionate intermediate pathway for thiosulfate oxidation in Acidithiobacillus caldus. 7**,** 1755.

Watts, S.F. (2000). The mass budgets of carbonyl sulfide, dimethyl sulfide, carbon disulfide and hydrogen sulfide. *Atmospheric Environment* 34**,** 761-779.

Williams, P.A., Coates, L., Mohammed, F., Gill, R., Erskine, P.T., Coker, A., Wood, S.P., Anthony, C., and Cooper, J.B. (2005). The atomic resolution structure of methanol dehydrogenase from *Methylobacterium extorquens*. *Acta Crystallographica Section D-Biological Crystallography* 61**,** 75-79.

Zhang, W., Li, F., and Nie, L. (2010). Integrating multiple 'omics' analysis for microbial biology: application and methodologies. *Microbiology* 156**,** 287-301.

Figures

Figure 1 Proposed pathways and reactions in *Methylophaga thiooxydans* DMS010 grown on DMS. Metabolic map showing selected pathways of *Methylophaga thiooxydans* DMS010. Metabolic pathways, both predicted from genome and proteomic analysis are presented in a simplified bacterial cell. Proteins detected in the proteomic analysis are indicated in light green and those not detected are indicated in white. Main metabolites are shown in square boxes and enzymes are shown in oval boxes. Some KEGG categories are indicated by color; green, one carbon pool by folate; blue, pentose phosphate path-ways (parts); orange, glycolysis; beige/brown, amino sugar and nucleotide sugar metabolism; purple, lipopolysaccharide biosynthesis; red, methane metabolism and yellow, sulfur metabolism. A solid arrow stands for an enzymatic reaction and a dashed arrow stands for multistep pathway. Abbreviations are explained in more detail in Supplementary Table S4; X? – uncharacterized DMS methyltransferase.

Figure 2 Simplified schematic overview of the proposed DMS degradation and subsequent route of sulfur oxidation in *Methylophaga thiooxydans* DMS010. Boden et al. (2010) suggested that DMS is initially degraded by a suggested cobalamin-dependent and as yet unidentified methyltransferase in the cytoplasm. Methanethiol (MT) would need to transfer to the periplasm where the methanethiol oxidase (Mto) degrades MT further as described in the text. The suggested steps of the sulfur oxidation pathway according to enzyme essays, proteomics and transcriptomics analyses are indicated. Abbreviations: MT, methanethiol; X?, unidentified putative methyltransferase; H_4F, tetrahydrofolate; CM, cytoplasmic membrane; OM, outer membrane; Mto; methanethiol oxidase; SuDH, sulfide dehydrogenase (flavocytochrome); SQR_p, periplasmatic sulfide:quinone reductase; SQR_c, cytoplasmatic sulfide:quinone reductase; Q, quinone, QH_2, quinol; SulP, sulfate permease; TauE, sulfite exporter; PDO, persulfide dioxygenase; TSDH, thiosulfate dehydrogenase; C_{550}, cytochrome 550 (located in the cytoplasmic membrane).

Figure 3 Pan-genome analysis of *Methylophaga* species. (A) Pan-genome tree consisting of six *Methylophaga* species was constructed using the neighbour-joining method within the EDGAR platform. **(B)** Number of core, dispensable, and specific genes (singletons) of each *Methylophaga* species. **(C)** Proportion of hypothetical and uncharacterized proteins in the core, dispensable and singleton genome of six *Methylophaga* species.

Figure 4 Metabolic pathways involved in DMS degradation/transformation, one-carbon and sulfur oxidation annotated with presence/absence of specific genes in the genomes of *Methylophaga*. The analysis was based on a six-way comparison among *M. thiooxydans* (T), *M. frappieri* (F), *M. aminisulfidivorans* (A), *M. lonarensis* (L), *M. nitratireducenticrescens* (N) and *M. sulfidovorans* (S). The shadings behind the letters indicate presence in core (light red) or dispensable (light blue) genome. The color-coded boxes next to the genes indicate the presence (green) or absence (orange) of a gene in each genome.

Supplementary Figure S1 SDS-PAGE of protein fractions of *Methylophaga thiooxydans* DMS010 grown on methanol or DMS. (A) Soluble protein fraction; lanes 1-3 *M. thiooxydans* DMS010 grown on methanol; lanes 4-6 *M. thiooxydans* DMS010 grown on DMS, S – Precision Plus Protein Standard (Bio-Rad Laboratories Inc.) (B) Membrane protein fraction; lanes 1-3 *M. thiooxydans* DMS010 grown on methanol; lanes 4-6 *M. thiooxydans* DMS010 grown on DMS, S – Precision Plus Protein Standard (Bio-Rad Laboratories Inc.)

Supplementary Figure S2 Schematic of the two gene clusters encoding methanethiol oxidases in Methylophaga thiooxydans. The locus tags (MDMS009_XXX) are indicated inside the arrows signifying the coding sequences, the annotation is indicated above the CDS as mtoX, methanethiol oxidase; sco1/mauG, SCO1/cytochrome c peroxidase MauG domain containing protein; tlpA, TlpA_like_family putative metallochaperone (conserved domain cd02966); pdo, putative persulfide dioxygenase; sqrB, sulfide quinone oxidoreductase; sulP, sulfate/sulfite_transporter; cysC, adenylylsulfate kinase; locus tag MDMS009_97 is annotated as a 'methylated-DNA--protein-cysteine methyltransferase'; rpoD, previously unannotated sigma70 factor. Shading indicates a that the CDS has a predicted signal peptide with a probability >50%, sqrB encoded by MDMS009_274 has a signal P likelihood of 12% (SignalP 5.0, (Armenteros et al., 2019); signal peptide predictions for MDS009_211 and MDMS009_894 are based on alternative start sites as discussed in the main text. Scale bar indicates 1 kb.

Supplementary Figure S3 Graphical summary of signal peptide predictions for the methanethiol oxidase encoded by MDMS009_211 contained on scaffold 1104571000268 (extended to positions 256503:257906). (A) Predicted amino acid sequence of the gene extended to the alternative upstream start codon. The underlined sequence indicates additional residues at N-terminal end, the blue highlighted sequence is in agreement with an experimentally determined N-terminal sequence obtained from a polypeptide expressed during

961 growth of *M. thiooxydans* on DMS (Schäfer, 2007). The SignalP 5.0 prediction graphical
962 summary and likelihood for signal peptide for the extended MDMS009_211 protein (B) and
963 the gene with the original start codon (C).
964
965 **Supplementary Figure S4 Graphical summary of signal peptide predictions for a**
966 **Sco1/SenC/PrrC domain protein predicted to be involved in cytochrome maturation**
967 **encoded by MDMS009_894 contained on scaffold 1104571000277 (extended to positions**
968 **41243:41737).** (A) Predicted amino acid sequence of the gene extended to an alternative
969 upstream start codon. The underlined sequence indicates additional residues at N-terminal end.
970 The SignalP 5.0 prediction graphical summary and likelihood for signal peptide for the
971 extended MDMS009_894 protein (B) and the gene with the original start codon (C).
972
973 **Supplementary Figure S5 Electrophoresis and electropherogram plots for evaluation of**
974 **purity and concentration of mRNA after enrichment from total RNA from *Methylophaga***
975 ***thiooxydans*.** RNA extraction and rRNA depletion led to successful mRNA enrichment. (a)
976 Electrophoresis plot showing one clear band for mRNA between 25 and 200 nucleotide (nt)
977 length in samples of *M. thiooxydans* grown on either methanol or DMS. (b to d)
978 Electropherogram plots showing mRNA of *M. thiooxydans* grown on methanol (replicates 1 to
979 3, respectively). (e to g) Electropherogram plots showing mRNA of *M. thiooxydans* grown on
980 DMS (replicates 1 to 3, respectively). mRNA concentrations are indicated in the plots and
981 ranged from 14.5 to 26.98 pg/µL in *M. thiooxydans* grown on methanol and from 85.7 to 172.0
982 pg/µL for *M. thiooxydans* grown on DMS.
983
984 **Supplementary Figure S6 Circular representation of the *M. thiooxydans* DMS010**
985 **chromosome including proteome and transcriptome expression data.** The first (innermost)
986 ring shows the expression (log2 ratio) of the proteome on DMS and methanol grown *M.*
987 *thiooxydans*, followed by the transcriptome expression (log2 ratio) data (second ring). The third
988 and sixth rings represent the COG categories according to (Tatusov et al., 2000). The fourth
989 and fifth rings represent the CDS (blue), tRNA (maroon), and rRNA (purple) on the reverse
990 and forward strand, respectively. The locations of several genes are indicated at the outside of
991 the map.
992
993 **Supplementary Figure S7 Average nucleotide identity (ANI) across the six different**
994 ***Methylophaga* species.**
995
996 ## Tables

Supplementary Table S1 Genome characteristics of the six *Methylophaga* genomes

Supplementary Table S2 List of query protein sequences used for genome comparison

Supplementary Table S3 Summary of protein fractions extracted from *Methylophaga thiooxydans* DMS010 grown in triplicates on either methanol or DMS.

Supplementary Table S4 Extract of proteins detected in *Methylophaga thiooxydans* DMS010 grown on DMS corresponding to Figure 2. Proteins with grey shading are up regulated in *Methylophaga thiooxydans* DMS010 grown on DMS compared to growth on methanol. Dark grey shadings indicate proteins up regulated more than 4-fold, light grey shadings indicate proteins up regulated between 1.3- to 4-fold. Selected proteins that were not detected in the proteome but are present in the genome of *Methylophaga thiooxydans* DMS0101 are included for clarity (marked with '-' in the last column Ratio DMS/Methanol).

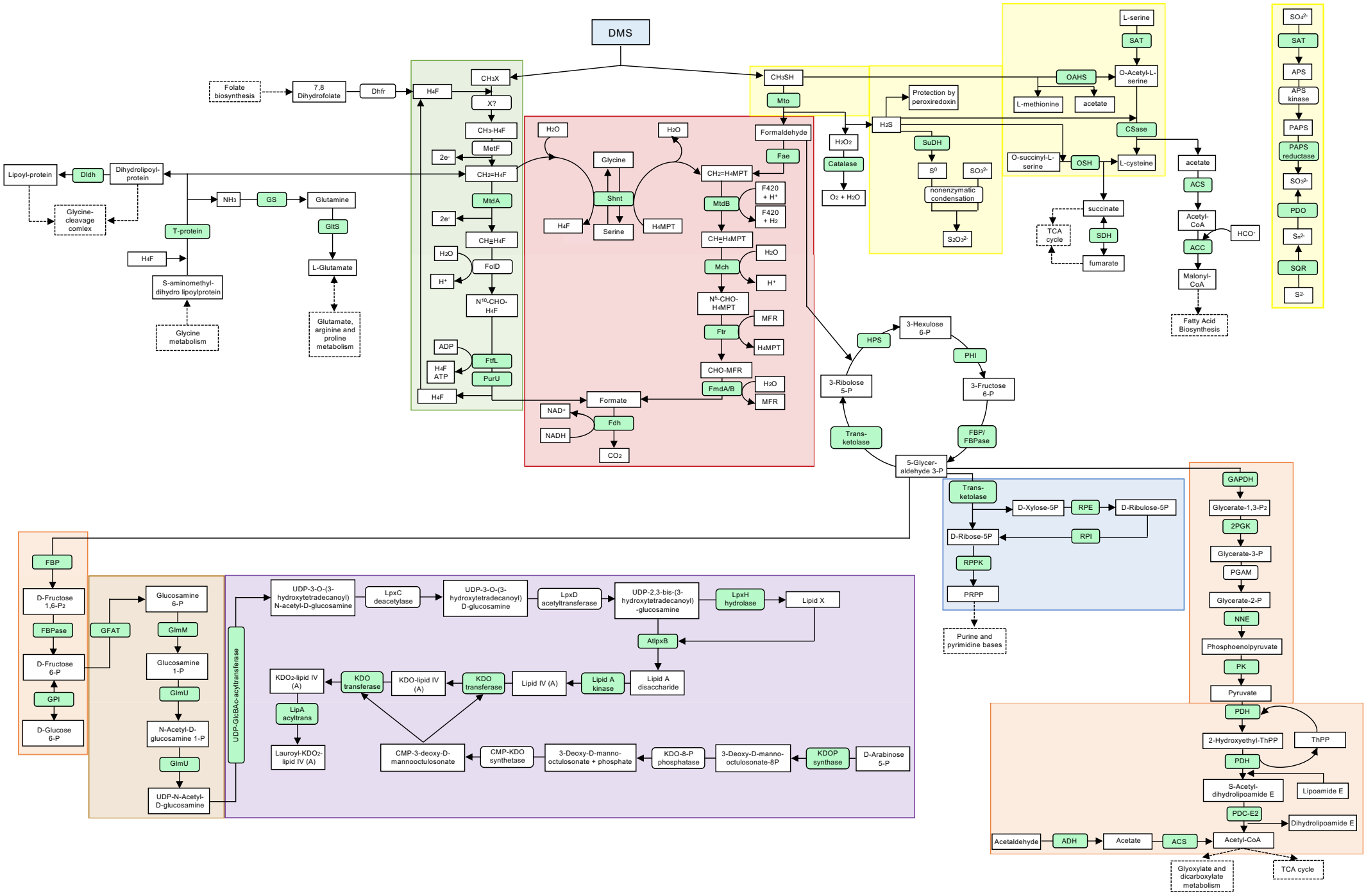
Supplementary Table S5 Hypothetical proteins detected as up-regulated in *Methylophaga thiooxydans* DMS010 grown on DMS compared to growth on methanol.

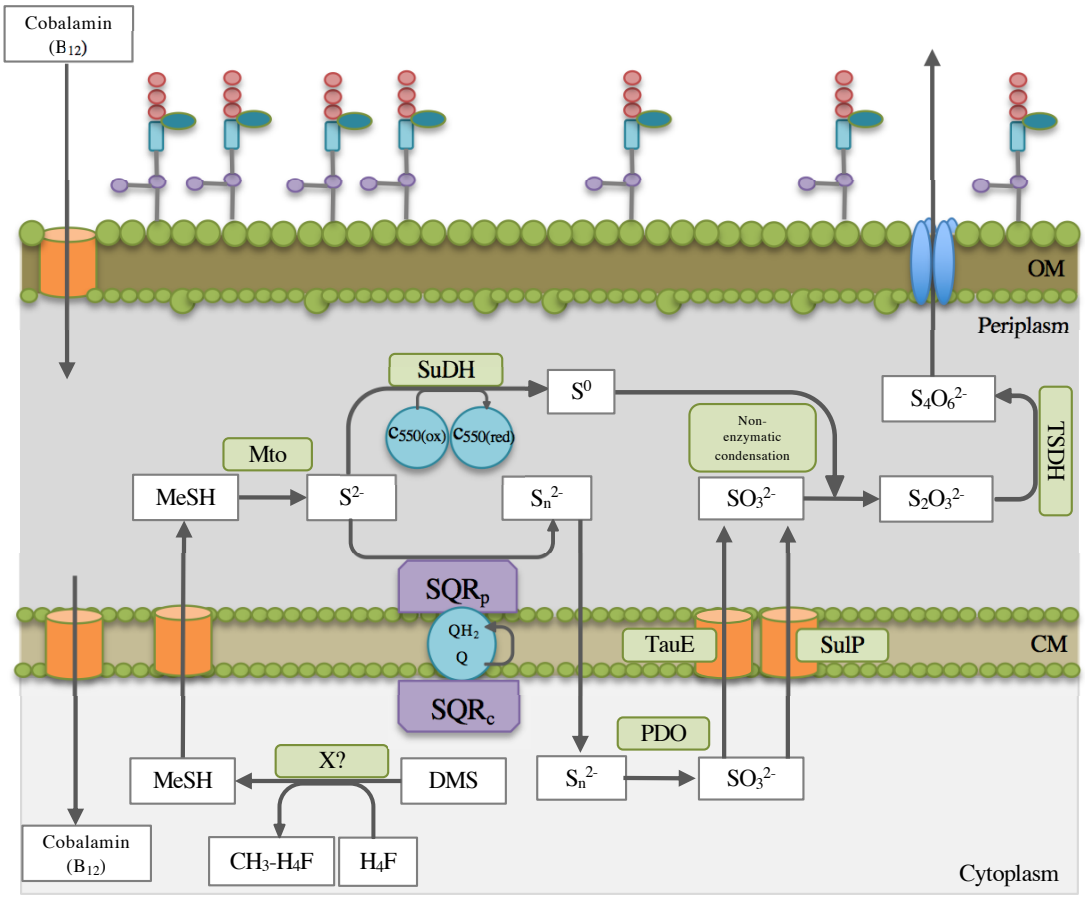
Supplementary Table S6 Comparison of transcript expression (mRNA) of *Methylophaga thiooxydans* DMS010 grown on methanol or DMS. Rockhopper calculates one expression level for each treatment (DMS/methanol) and each transcript from the triplicate RNA sequencing results as explained in the text above.

Supplementary Table S7 Characteristics of six *Methylophaga* species

Supplementary Table S8 Presence and absence of genes involved in DMS degradation and sulfur metabolism in six *Methylophaga* species

Supplementary Table S9 Previously unannotated and/or not predicted genes in *Methylophaga thiooxydans*.

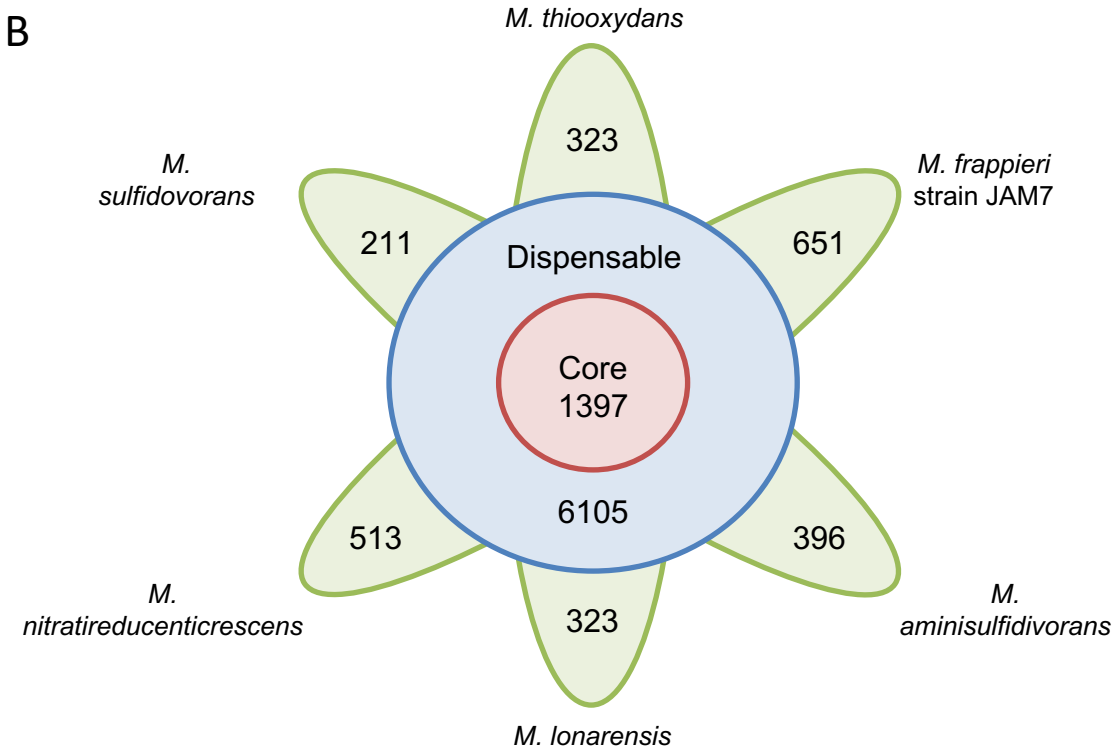

DMS
CH3SH
Mto
H2S
Protection by peroxiredoxin
SuDH
S0
SO32-
nonenzymatic condensation
S2O32-
H2O2
Catalase
O2 + H2O
L-serine
SAT
O-Acetyl-L-serine
OAHS
L-methionine
acetate
CSase
L-cysteine
O-succinyl-L-serine
OSH
succinate
SDH
fumarate
TCA cycle
acetate
ACS
Acetyl-CoA
HCO3-
ACC
Malonyl-CoA
Fatty Acid Biosynthesis
SO42-
SAT
APS
APS kinase
PAPS
PAPS reductase
SO32-
PDO
Sn2-
SQR
S2-
Folate biosynthesis
7,8 Dihydrofolate
Dhfr
H4F
CH3X
X?
CH3-H4F
MetF
2e-
CH2=H4F
MtdA
CH≡H4F
H2O
FolD
H+
N10-CHO-H4F
ADP
H4F ATP
FtfL
PurU
Formate
NAD+
NADH
Fdh
CO2
Glycine
Shnt
Serine
H4MPT
Formaldehyde
Fae
CH2=H4MPT
MtdB
F420 + H+
F420 + H2
CH≡H4MPT
Mch
N5-CHO-H4MPT
Ftr
MFR
CHO-MFR
FmdA/B
Lipoyl-protein
Dldh
Dihydrolipoyl-protein
Glycine-cleavage comlex
NH3
GS
Glutamine
GltS
L-Glutamate
Glutamate, arginine and proline metabolism
T-protein
S-aminomethyl-dihydro lipoylprotein
Glycine metabolism
3-Hexulose 6-P
HPS
PHI
3-Fructose 6-P
3-Ribolose 5-P
Trans-ketolase
FBP/ FBPase
5-Glycer-aldehyde 3-P
Trans-ketolase
D-Xylose-5P
RPE
D-Ribulose-5P
D-Ribose-5P
RPI
RPPK
PRPP
Purine and pyrimidine bases
GAPDH
Glycerate-1,3-P2
2PGK
Glycerate-3-P
PGAM
Glycerate-2-P
NNE
Phosphoenolpyruvate
PK
Pyruvate
PDH
2-Hydroxyethyl-ThPP
ThPP
PDH
S-Acetyl-dihydrolipoamide E
Lipoamide E
PDC-E2
Dihydrolipoamide E
Acetaldehyde
ADH
Acetate
ACS
Acetyl-CoA
Glyoxylate and dicarboxylate metabolism
TCA cycle
FBP
D-Fructose 1,6-P2
FBPase
D-Fructose 6-P
GPI
D-Glucose 6-P
GFAT
Glucosamine 6-P
GlmM
Glucosamine 1-P
GlmU
N-Acetyl-D-glucosamine 1-P
GlmU
UDP-N-Acetyl-D-glucosamine
UDP-GlcNAc-acyltransferase
UDP-3-O-(3-hydroxytetradecanoyl) N-acetyl-D-glucosamine
LpxC deacetylase
UDP-3-O-(3-hydroxytetradecanoyl) D-glucosamine
LpxD acetyltransferase
UDP-2,3-bis-(3-hydroxytetradecanoyl) -glucosamine
LpxH hydrolase
Lipid X
AtlpxB
Lipid A disaccharide
Lipid A kinase
Lipid IV (A)
KDO transferase
KDO-lipid IV (A)
KDO transferase
KDO2-lipid IV (A)
LipA acyltrans
Lauroyl-KDO2-lipid IV (A)
CMP-3-deoxy-D-mannooctulosonate
CMP-KDO synthetase
3-Deoxy-D-manno-octulosonate + phosphate
KDO-8-P phosphatase
3-Deoxy-D-manno-octulosonate-8P
KDOP synthase
D-Arabinose 5-P


Cobalamin
(B12)
OM
Periplasm
SuDH
S0
S4O62-
C550(ox)
C550(red)
Non-
enzymatic
condensation
TSDH
Mto
MeSH
S2-
Sn2-
SO32-
S2O32-
SQRp
QH2
Q
TauE
SulP
CM
SQRc
PDO
X?
MeSH
DMS
Sn2-
SO32-
Cobalamin
(B12)
CH3-H4F
H4F
Cytoplasm

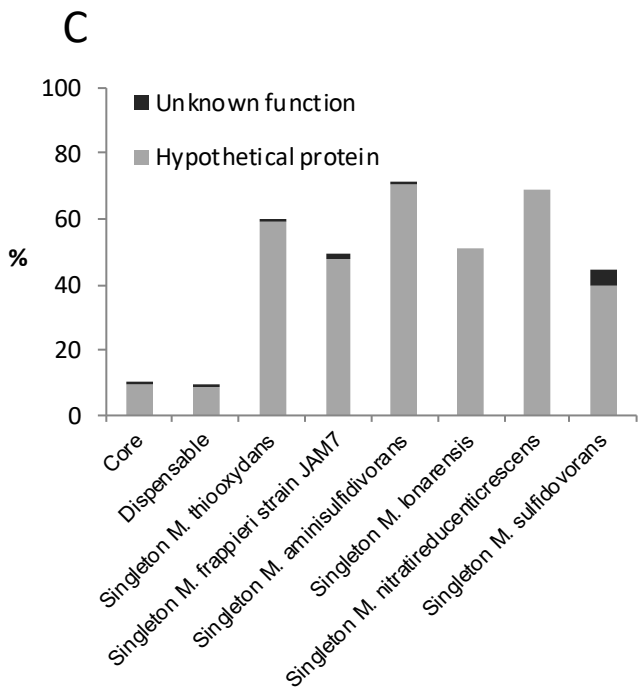
A

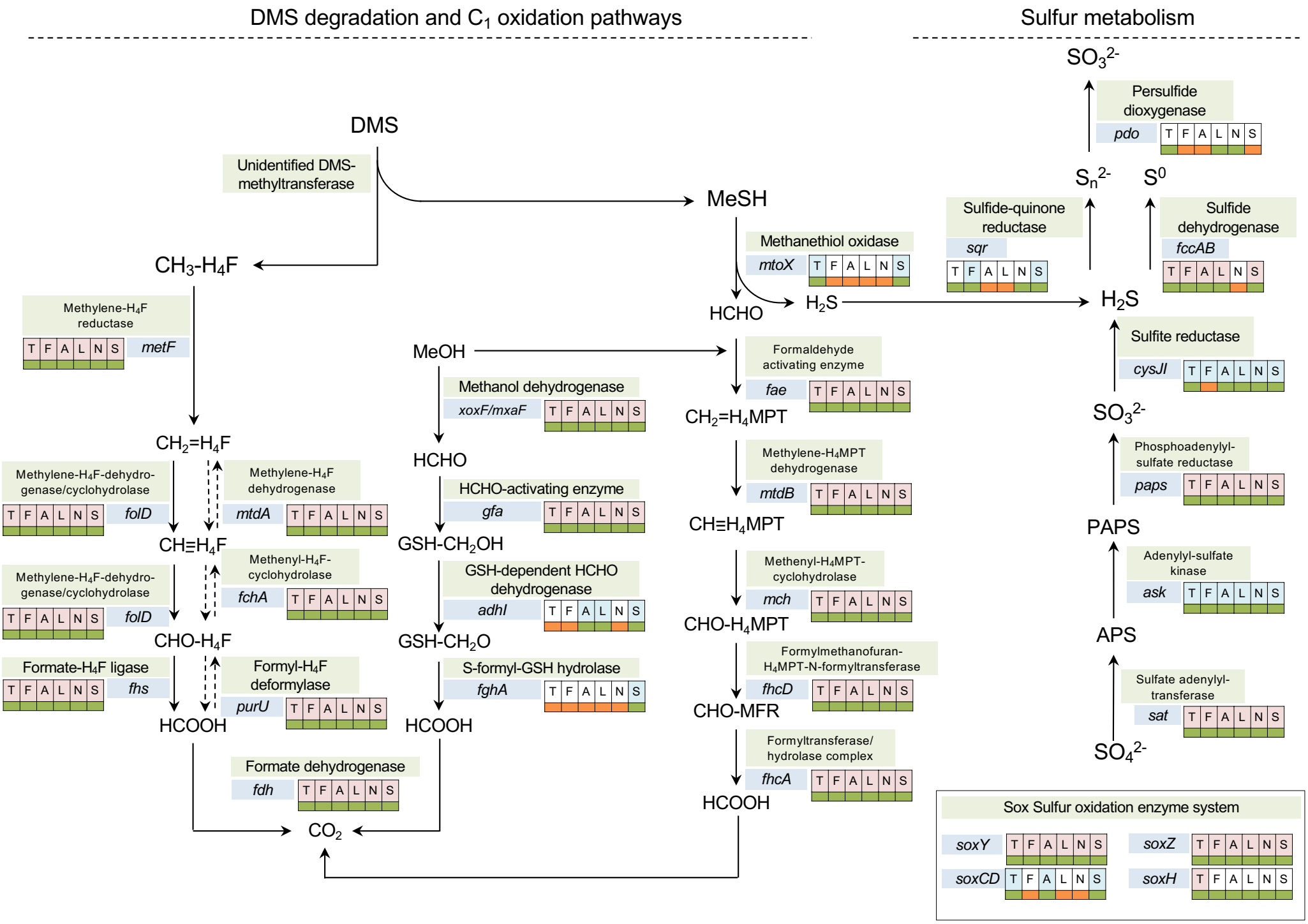

M. nitratireducenticrescens
M. frappieri strain JAM7
M. aminisulfidivorans
M. sulfidovorans
M. thiooxydans
M. lonarensis
0.01

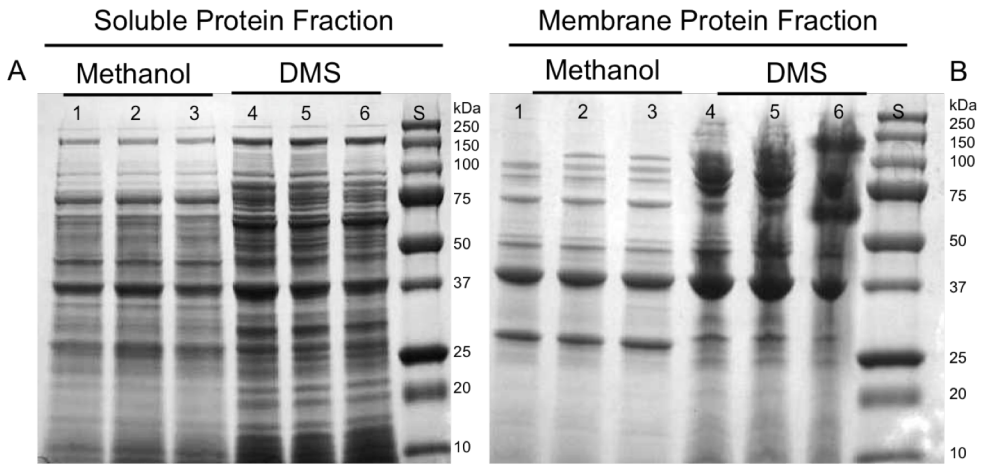
B


M. thiooxydans
323
M. sulfidovorans
211
M. frappieri strain JAM7
651
Dispensable
Core 1397
6105
513
396
M. nitratireducenticrescens
323
M. aminisulfidivorans
M. lonarensis

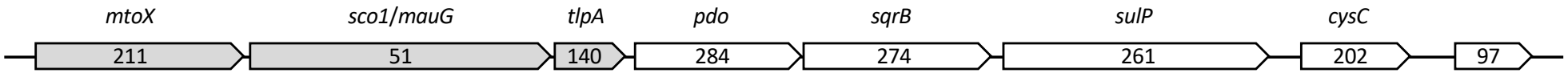
C


100
80
60
40
20
0
%
Unknown function
Hypothetical protein
Core
Dispensable
Singleton M. thiooxydans
Singleton M. frappieri strain JAM7
Singleton M. aminisulfidivorans
Singleton M. lonarensis
Singleton M. nitratireducenticrescens
Singleton M. sulfidovorans

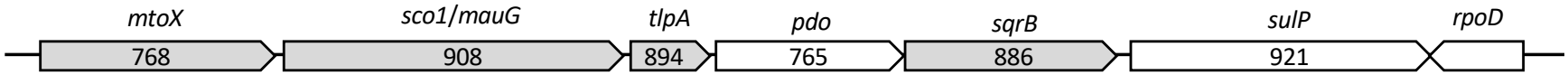

DMS degradation and C1 oxidation pathways
Sulfur metabolism
DMS
Unidentified DMS-methyltransferase
MeSH
Methanethiol oxidase
mtoX
HCHO
H2S
CH3-H4F
Methylene-H4F reductase
metF
CH2=H4F
Methylene-H4F-dehydrogenase/cyclohydrolase
folD
Methylene-H4F dehydrogenase
mtdA
CH≡H4F
Methenyl-H4F-cyclohydrolase
fchA
CHO-H4F
Formate-H4F ligase
fhs
Formyl-H4F deformylase
purU
HCOOH
MeOH
Methanol dehydrogenase
xoxF/mxaF
HCHO
HCHO-activating enzyme
gfa
GSH-CH2OH
GSH-dependent HCHO dehydrogenase
adhI
GSH-CH2O
S-formyl-GSH hydrolase
fghA
HCOOH
Formate dehydrogenase
fdh
CO2
Formaldehyde activating enzyme
fae
CH2=H4MPT
Methylene-H4MPT dehydrogenase
mtdB
CH≡H4MPT
Methenyl-H4MPT-cyclohydrolase
mch
CHO-H4MPT
Formylmethanofuran-H4MPT-N-formyltransferase
fhcD
CHO-MFR
Formyltransferase/hydrolase complex
fhcA
HCOOH
Sulfide-quinone reductase
sqr
Persulfide dioxygenase
pdo
SO3 2-
Sn 2-
S0
Sulfide dehydrogenase
fccAB
H2S
Sulfite reductase
cysJI
SO3 2-
Phosphoadenylyl-sulfate reductase
paps
PAPS
Adenylyl-sulfate kinase
ask
APS
Sulfate adenylyl-transferase
sat
SO4 2-
Sox Sulfur oxidation enzyme system
soxY
soxZ
soxCD
soxH
T F A L N S


Soluble Protein Fraction
Membrane Protein Fraction
A
B
Methanol
DMS
Methanol
DMS
1 2 3 4 5 6 S
1 2 3 4 5 6 S
kDa
250
150
100
75
50
37
25
20
10
kDa
250
150
100
75
50
37
25
20
10

mto-cluster 1



mto-cluster 2

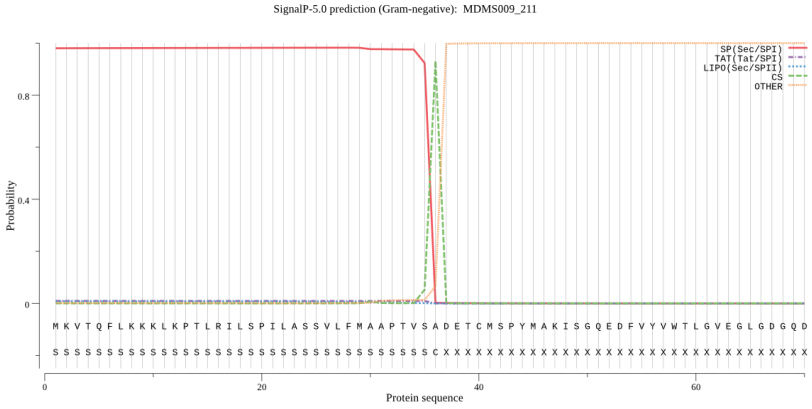


1 kb

A

>MDMS009_211 extended scf_1104571000268; 256503:257906
MKVTQFLKKKLKPTLRILSPILASSVLFMAAPTVSADETCMSPYMAKISGQEDFVYVWTL
GVEGLGDGQDKLVTVSVNPEADDYGKVRHVTSVGGRNEAHHSGFTDDRKYLWAGGLDTNK
IFIFDVYSDPAKPTLVKTIDDFVSKTGGVVGPHTPYALPGRMLWTALSNNQDHGGRTALV
EYTNEGEYVDTYWIPTDDNLQGAEKTGQYADGYGYDIQVLPRRNMMLTSSFTGWSNYMMD
FGQMLQDKEAMKRFGNTMVVWDLHTRQPKQVLDVEGAPLEIRCAWGAENNYCFTITALTA
EIVLIEEDDKGGWKAEVVGTVANPSDVPLPVDFSISSDDSMIWVNTFMDGTTRGYDITDP
HNPKLAHEQYIGEQINMVSSSWDNKRLYYTTSLLANWDHKGEKDVQFMKLMHWDPETKTM
EEQFHIDFYKEKLGRAHQMRFGAYSLYGKQAKHESDADAQLALTQEK

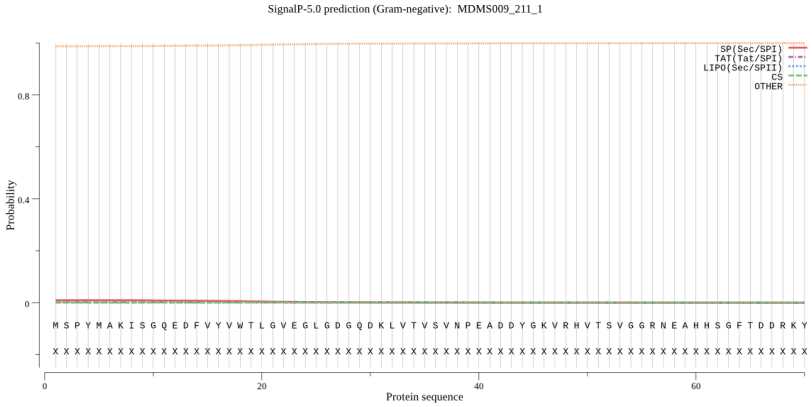
B



Signal-P 5.0 analysis of extended CDS of MDMS009_211 encoding methanethiol oxidase

Signal peptide likelihood Sec/SPI: 0.9819

C



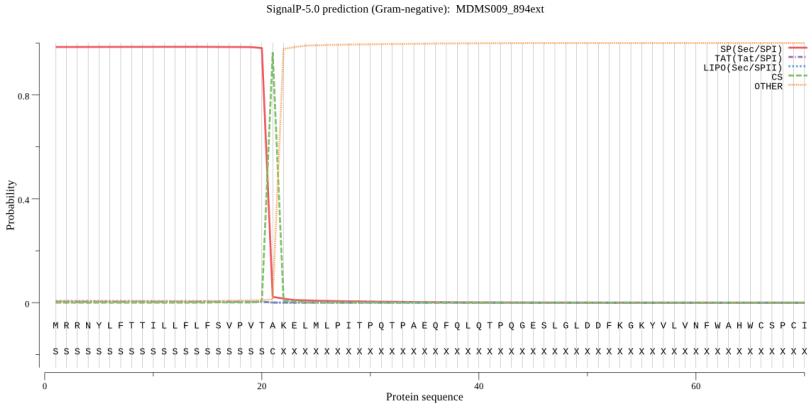
Signal-P 5.0 analysis of Original predicted CDS of MDMS009_211 Encoding methanethiol oxidase

Signal peptide likelihood Sec/SPI: 0.0096

A

>MDMS009_894 extended scf_1104571000277 - 41243: 41737
MRRNYLFTTILLFLFSVPVTAKELMLPITPQTPAEQFQLQTPQGESLGLDDFKGKYVLVN
FWAHWCSPCIKEFPDMQRLYEQTDRERFEIIGIHAGPYTTEAAEFITHFGITFPVVSDPD
TSLKGWNVKALPMTYLVNPEGQLIYEAVGPREWDFQEMKALITN

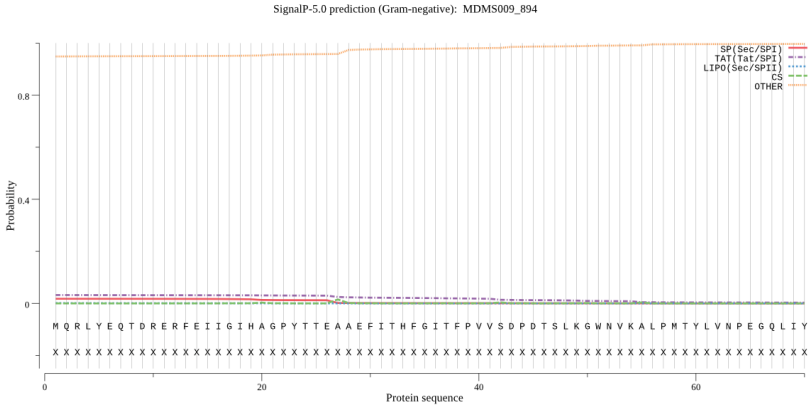
B



Signal-P 5.0 analysis of extended CDS of MDMS009_894

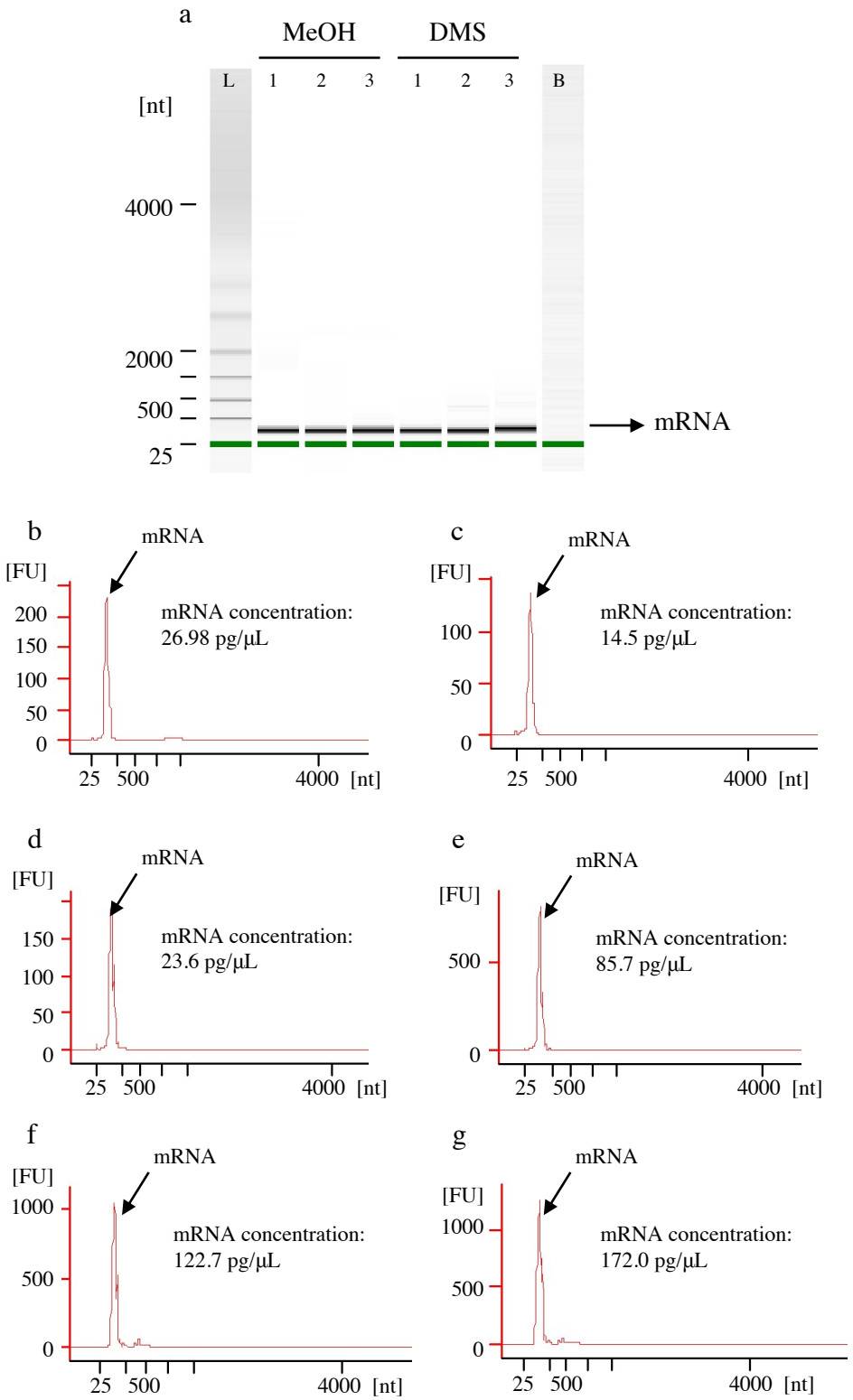
Signal peptide likelihood Sec/SPI: 0.9847

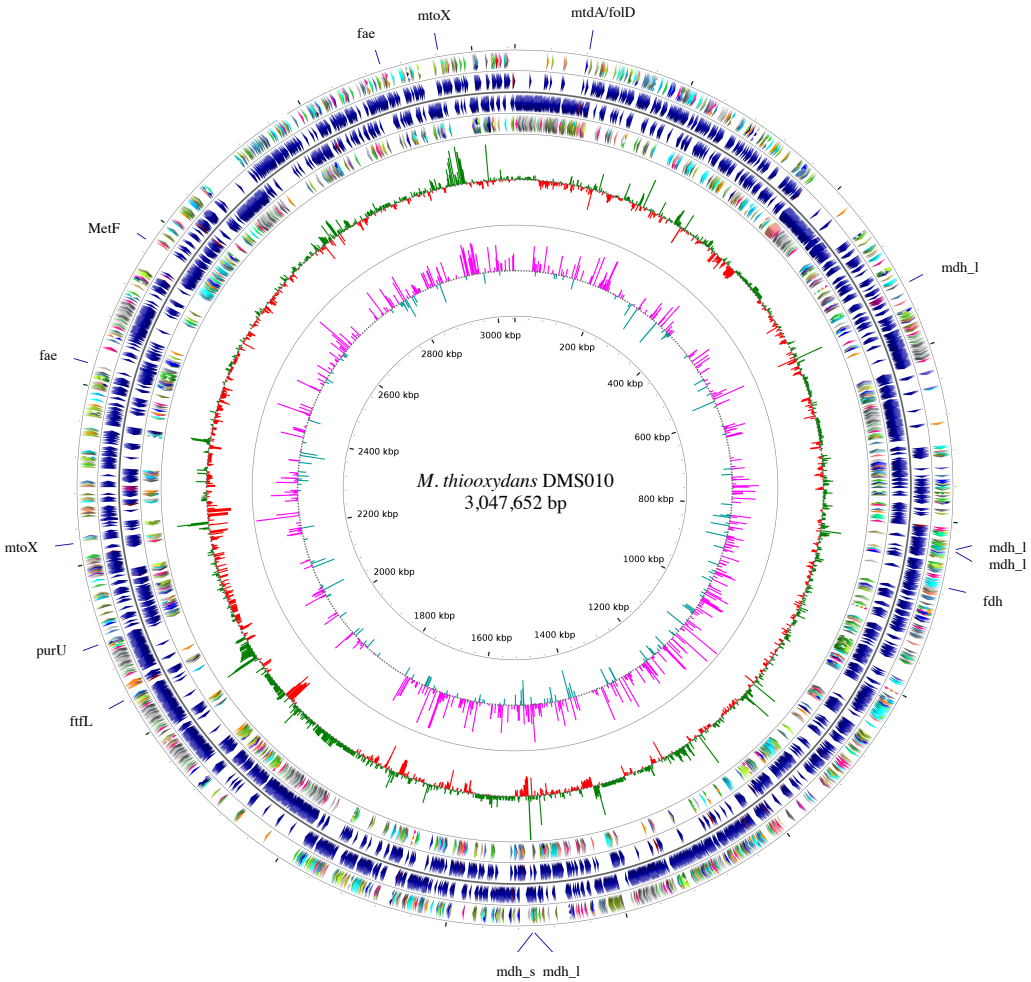
C



Signal-P 5.0 analysis of Original predicted CDS of MDMS009_894

Signal peptide likelihood Sec/SPI: 0.018


a
MeOH
DMS
L
1
2
3
1
2
3
B
[nt]
4000
2000
500
25
mRNA
b
mRNA
[FU]
200
150
100
50
0
mRNA concentration:
26.98 pg/µL
25
500
4000
[nt]
c
mRNA
[FU]
100
50
0
mRNA concentration:
14.5 pg/µL
25
500
4000
[nt]
d
mRNA
[FU]
150
100
50
0
mRNA concentration:
23.6 pg/µL
25
500
4000
[nt]
e
mRNA
[FU]
500
0
mRNA concentration:
85.7 pg/µL
25
500
4000
[nt]
f
mRNA
[FU]
1000
500
0
mRNA concentration:
122.7 pg/µL
25
500
4000
[nt]
g
mRNA
[FU]
1000
500
0
mRNA concentration:
172.0 pg/µL
25
500
4000
[nt]



Ring Legend (from the center)

1) Proteome expression

- Upregulated on DMS
- Upregulated on methanol

2) Transcriptome expression

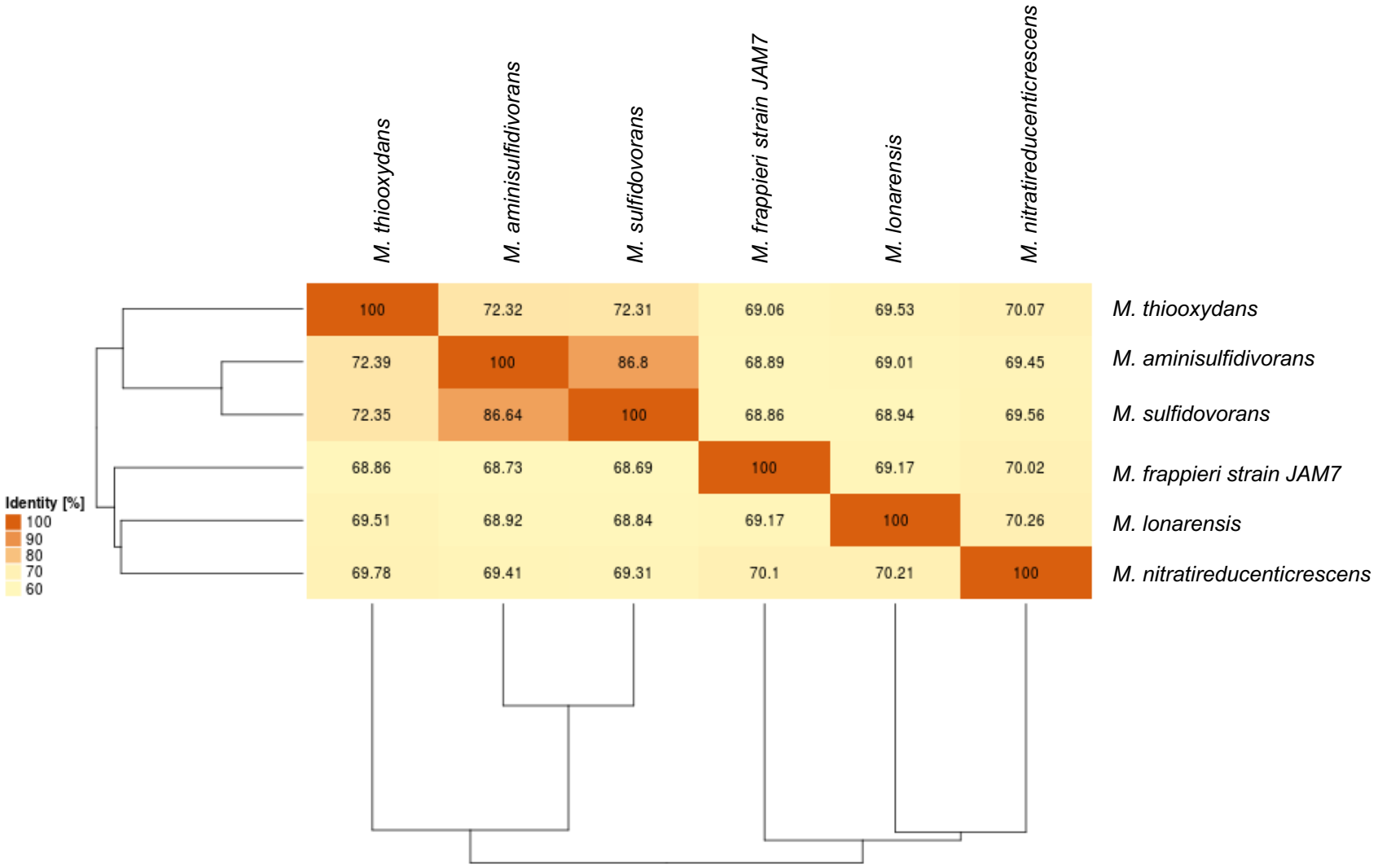
- Upregulated on DMS
- Upregulated on methanol

3) and 6) COG categories

A	D	T	Z	H
B	O	U	C	I
J	M	V	G	Q
K	N	W	E	R
L	P	Y	F	S

4) and 5) Features

- CDS
- tRNA
- rRNA


M. thiooxydans
M. aminisulfidivorans
M. sulfidovorans
M. frappieri strain JAM7
M. lonarensis
M. nitratireducenticrescens
100 72.32 72.31 69.06 69.53 70.07
72.39 100 86.8 68.89 69.01 69.45
72.35 86.64 100 68.86 68.94 69.56
68.86 68.73 68.69 100 69.17 70.02
69.51 68.92 68.84 69.17 100 70.26
69.78 69.41 69.31 70.1 70.21 100
M. thiooxydans
M. aminisulfidivorans
M. sulfidovorans
M. frappieri strain JAM7
M. lonarensis
M. nitratireducenticrescens
Identity [%]
100
90
80
70
60