The deep-subsurface sulfate reducer *Desulfotomaculum kuznetsovii* employs two methanol-degrading pathways

Diana Z. Sousa, Michael Visser, Antonie H. van Gelder, Sjef Boeren, Mervin M. Pieterse, Martijn W.H. Pinkse, Peter D.E.M. Verhaert, Carsten Vogt, Steffi Franke, Steffen Kümmel & Alfons J.M. Stams

Methanol is generally metabolized through a pathway initiated by a cobalamine-containing methanol methyltransferase by anaerobic methylotrophs (such as methanogens and acetogens), or through oxidation to formaldehyde using a methanol dehydrogenase by aerobes. Methanol is an important substrate in deep-subsurface environments, where thermophilic sulfate-reducing bacteria of the genus *Desulfotomaculum* have key roles. Here, we study the methanol metabolism of *Desulfotomaculum kuznetsovii* strain 17T, isolated from a 3000-m deep geothermal water reservoir. We use proteomics to analyze cells grown with methanol and sulfate in the presence and absence of cobalt and vitamin B12. The results indicate the presence of two methanol-degrading pathways in *D. kuznetsovii*, a cobalt-dependent methanol methyltransferase and a cobalt-independent methanol dehydrogenase, which is further confirmed by stable isotope fractionation. This is the first report of a microorganism utilizing two distinct methanol conversion pathways. We hypothesize that this gives *D. kuznetsovii* a competitive advantage in its natural environment.
Hig\textit{h} temperatures and oligotrophic conditions often prev\textit{al} in deep-subsurface environments, which can be useful for underground gas storage and geothermal energy production\textsuperscript{1}. However, the resident microbial communities influence possible applications, and these in turn affect the ecology of the deep-subsurface microbiota. Therefore, understanding the microbial composition of deep-subsurface environments and the metabolism of their community members is important. Studies so far showed a dominance of Gram-positive, spore forming, thermophilic bacteria in high-temperature subsurface environments, especially \textit{Desulfotomaculum} species\textsuperscript{2-5}. Many \textit{Desulfotomaculum} species are thermophilic and can grow in vitamin-deprived environments\textsuperscript{6,7}. They possess a rather versatile metabolism and their spores are extremely heat resistant\textsuperscript{8,9}, which make them perfectly adapted to subsurface conditions.

Methanol is an important substrate for microbial life in deep-subsurface environments\textsuperscript{10,11}. Methanol is a common compound in nature and it is naturally produced by the degradation of pectin and lignin, which are constituents of plant cell walls\textsuperscript{12}. However, in the deep-subsurface methanol may be geochemically produced from \textit{CO}_2 and \textit{H}_2, a gas mixture commonly present in these environments due to the geological production of hydrogen. Abiotic synthesis of methanol in conditions characteristic for deep-subsurface environments was described\textsuperscript{13}.

Several phylogenetic groups of microorganisms are able to grow with methanol as a sole carbon and energy source. Aerobic and facultative anaerobic methylo trophs generally convert methanol to formaldehyde by a methanol dehydrogenase (MDH). Multiple MDHs, such as MDHs that use pyrroloquinoline quinone (PQQ) or NAD(P) as a cofactor, have been characterized\textsuperscript{14,15}. Recently, two types of PQQ-dependent MDHs were described to be present in \textit{Methylobacterium extorquens} AM1. A PQQ MDH using calcium in its active site and another using lanthanides\textsuperscript{16}. In anoxic deep-subsurface environments methylo trophs such as methanogenic archaea, acetogenic bacteria, and sulfate-reducing bacteria compete for methanol. Methanogens and acetogens employ a methanol methyltransferase (MT) system\textsuperscript{17-23}. This system involves two enzymes, \textit{MT}_1 and \textit{MT}_2. \textit{MT}_1 consists of two subunits, the first (\textit{MtaB}) is involved in breaking the C=O bond of methanol and transferring the methyl residue to the second subunit (\textit{MtaC}). \textit{MT}_2 (\textit{MtaA}) transfers the methyl group from \textit{MtaC} to coenzyme M in methanogens\textsuperscript{17-20}, or tetrahydrofolate in acetogens\textsuperscript{21-23}.

The methanol metabolism of sulfate-reducing bacteria (SRB) has not been extensively studied. It is not clear whether SRB use a MT system or a MDH. Several SRB utilize methanol for growth, such as \textit{Desulfosporosinus orientis}\textsuperscript{24}, \textit{Desulfbacterium catechlicom}\textsuperscript{25}, \textit{Desulfbacterium anilini}\textsuperscript{26}, \textit{Desulfovibrio carbinolicus}\textsuperscript{27}, \textit{Desulfovibrio alcoholivorans}\textsuperscript{28}, and nine \textit{Desulfotomaculum} strains\textsuperscript{10,29-33} including \textit{D. kuznetsovii}. The latter species is a methylocrophic thermophilic sulfate-reducing bacterium that was isolated from a geothermal water reservoir at a depth of about 3000 m\textsuperscript{10}. We studied the metabolism of this sulfate reducer to get insight into its growth strategy in oligotrophic deep-subsurface environments. Growth of \textit{D. kuznetsovii} with methanol and sulfate was studied and resulted in a partially purified alcohol dehydrogenase (ADH) with a molecular mass of 42 kDa that also showed activity with methanol, but activity with ethanol was ten times higher\textsuperscript{34}. Analysis of the genome of \textit{D. kuznetsovii} revealed the putative presence of methanol methyltransferase genes as well\textsuperscript{7}. Therefore, the methanol metabolism in \textit{D. kuznetsovii} remained unsolved and we hypothesized that the bacterium possesses two distinct methanol-degradation pathways, which has never been described in other microorganisms.

Here we show evidence for the presence of two methanol-degradation pathways in \textit{D. kuznetsovii} by analyzing the proteome of cells grown with methanol and sulfate in the presence and absence of cobalt and vitamin B12. Importantly, stable isotope fractionation analysis of cells grown in media with cobalt and vitamin B12 indicates that during growth the alcohol dehydrogenase is used first and the MT is operating later at lower methanol concentrations.

Results

\textbf{Effect of cobalt and vitamin B12 on growth with methanol.} The presence of genes coding for a methanol MT system in the genome of \textit{D. kuznetsovii} suggested the involvement of a vitamin B12-dependent MT system in methanol conversion\textsuperscript{7}, while previous analysis indicated the involvement of an alcohol dehydrogenase\textsuperscript{34}. To clarify the role of these enzyme systems we assessed the effect of cobalt on growth with methanol.

When cobalt and vitamin B12 were omitted from the medium \textit{D. kuznetsovii} was still able to degrade methanol, but the residual methanol concentration at the end of the assays was significantly higher (\textit{p} = 0.00027) than in assays with cobalt and vitamin B12 (Supplementary Fig. 1). This indicates the presence of a second, cobalamin-independent, methanol-degradation pathway, and suggests the importance of the methanol MT system for the conversion of low concentrations of methanol.

\textbf{Comparative proteomics shows two methanol-degrading pathways.} \textit{D. kuznetsovii} cells were adapted to four different growth conditions: methanol and sulfate in presence and absence of cobalt and vitamin B12, lactate and sulfate, and ethanol and sulfate. The lactate growth condition was used as a reference, whereas the ethanol growth condition was used because previous research indicated the involvement of an alcohol dehydrogenase for growth with methanol and ethanol\textsuperscript{34}. Protein abundance data under the different conditions are shown in Supplementary Data 1, and the abundance of physiologically important proteins involved in methanol metabolism is visualized in Fig. 1. Assays with methanol were performed using initial substrate concentrations of 20 mM and 5 mM, but main results and trends were similar for both conditions (for this reason results from assays with 5 mM methanol are omitted in the manuscript and provided only in Supplementary Data 1).

Growth of \textit{D. kuznetsovii} with methanol in the presence of cobalt and vitamin B12 resulted in increased abundance of proteins encoded by genes of an operon (Desku_0050-60), which were annotated as proteins involved in vitamin B12 biosynthesis and a predicted methanol MT system (Fig. 1; Supplementary Data 1). Two MtaA MTs, a MtaB and MtaC are highly abundant under these conditions. The increased abundance of the corrinoid binding MtaC indicates the necessity of vitamin B12 in the cell. No vitamin B12 transport encoding genes were found in the genome of \textit{D. kuznetsovii} and all genes essential for vitamin B12 synthesis were present in the genome\textsuperscript{2}. Only vitamin B12 synthesis proteins encoded by genes of the operon structure Desku_0050-0060 were more abundant during growth with methanol and cobalt, which coincides with the higher expression of the MT system in these conditions.

In other studies, cobalt limitation led to decreased conversion rates of methanogens and acetogens when grown with methanol\textsuperscript{25-38}. This was explained by the essential role of cobalt in corrinoid biosynthesis\textsuperscript{38} and the synthesis of corrinoid-dependent proteins by the methanol utilization\textsuperscript{20,35-37,39}. The MtaC subunit of the methanol MT system was described to bind the corrinoid\textsuperscript{21,40,41}. When cobalt and vitamin B12 were omitted from the medium the abundance of the MT system and the vitamin B12 synthesis pathway were very low (Fig. 1). Growth on methanol (with and without cobalt and vitamin B12) and on
ethanol resulted in high abundance of an alcohol dehydrogenase (Desku_2952) and an aldehyde ferredoxin oxidoreductase (Desku_2951) (Fig. 1), indicating the involvement of those proteins in both the methanol and ethanol metabolism of D. kuznetsovi.

Goorissen partially purified an ADH with a molecular mass of 42 kDa that showed activity with ethanol and methanol\[^{34}\]. In that study, the ADH was present during growth with ethanol and sulfate. However, the ADH activity with ethanol was ten times higher than with methanol. Activity could be measured with nicotinamide adenine dinucleotide (NAD), 2,6 dichlorophenolindophenol (DCPIP), and 3-(4,5-dimethylthiazol-2-yl)-2,4 diphenyltetrazolium bromide (MTT), but not with nicotinamide

![Volcano plot](image-url)
adenine dinucleotide phosphate (NADP). The highest activity was measured with ethanol and NAD. Moreover, activity of the reverse reaction was measured when using both acetaldehyde and formaldehyde.

Our results indicate that the partially purified ADH described by Goorissen is the Desk_u_2952 ADH. In agreement with that study the abundance of the Desk_u_2952 ADH is higher when cells were grown with methanol compared to ethanol-grown cells (Fig. 1b) and the predicted size of the Desk_u_2952 ADH is 41 kDa. Two other alcohol dehydrogenases (Desk_u_0619, 3082) and four other aldehyde dehydrogenases (Desk_u_0621, 2946, 2983, 3081) were identified in the genome and some in proteome data (Supplementary Data 1), but these did not exhibit enhanced abundance in any of the growth conditions that we tested or any abundance at all. Therefore, they do not seem to be specifically involved in the ethanol and/or methanol degradation. These results suggest that two methanol-utilizing pathways are present in *D. kuznetsovii* as visualized in Fig. 2.

The MtaB (Desk_u_0051) and the ADH (Desk_u_2952) amino acid sequences and closely related protein sequences of other microorganisms were used to generate phylogenetic trees (Figs. 3 and 4). Figure 3 shows the distribution of MtaB proteins of sulfate reducers, acetogens, and methanogens. Interestingly, the phylogenetic tree displays two major clades where *D. kuznetsovii* resides in the same clade as methanogens, while other Gram-positive SRB, like *Desulfosporosinus* species, cluster together with acetogens in the other clade. This leads to the suggestion that the MT system of *D. kuznetsovii* is evolutionarily closer to the MT system of methanogens than to that of acetogens, which is a remarkably unexpected finding. This could be due to a horizontal gene transfer event.

*D. kuznetsovii* has six ADH encoding genes in its genome, which cluster separately in an amino acid sequence neighbor-joining tree (Fig. 4). This suggests that their sequences differ from each other, which could coincide with different substrate specificity. Interestingly, the methanol-oxidizing ADH clusters together with ADH sequences of species that are able to use ethanol, but are unable or not known to utilize methanol.

Stable isotope fractionation analysis. The proteomics data showed that enzymes of the two methanol-degrading pathways are produced when *D. kuznetsovii* is grown with methanol and sulfate in the presence of cobalt. To assess the contribution of each pathway under these conditions we performed a compound specific stable carbon isotope analysis. The methyltransferase reaction has been shown to result in a large stable carbon isotope fractionation upon methanol conversion to methane by methanogens42,43. No data are available for carbon isotope fractionation upon methanol conversion to methane by methanogens42,43. No data are available for carbon isotope fractionation upon methanol conversion to methane by methanogens. However, we have shown that *D. kuznetsovii* is able to utilize methanol, and that the methanol-oxidizing ADH clusters with ADH sequences of species that are able to use ethanol, but are unable or not known to utilize methanol.

---

**Fig. 2** Hypothesized methanol metabolism pathways in *D. kuznetsovii*. Methanol is oxidized to CO$_2$ by an alcohol dehydrogenase (ADH), aldehyde ferredoxin oxidoreductase (AFO), and a formate dehydrogenase (FDH). When cobalt is present in the environment a second concurrent methanol-oxidizing pathway is induced and part of the methanol is methylated to methyl-tetrahydrofolate (CH$_3$-THF). Subsequently, CH$_3$-THF is oxidized to CO$_2$ generating the same amount of electrons. Locus tag numbers are indicated for boxed enzymes.

**Fig. 3** Neighbor-joining tree based on MtaB amino acid sequences. The sequences were obtained from a BLASTp analysis, using MtaB of *D. kuznetsovii* as the query sequence. MtaB of *D. kuznetsovii* is printed in bold. Closed circles represent bootstrap values of 75% or higher. Scale bar represents 10% sequence difference.
were grown with methanol and sulfate in the presence and absence of cobalt and vitamin B12. The percentage of degraded methanol was measured during the course of methanol degradation, indicating that methanol oxidation by the ADH is indeed not associated to a carbon isotope effect.

These results show that in the medium without cobalt, the condition in which *D. kuznetsovii* predominantly synthesized the methanol-oxidizing ADH, no significantly fractionation occurs. In the medium with cobalt, the condition in which *D. kuznetsovii* also synthesized the methanol methyltransferases, considerable fractionation was observed. As can be seen in the double-logarithmic Rayleigh plot (Supplementary Fig. 2), in the medium with cobalt isotope fractionation started to occur after a certain amount of methanol was degraded. This strongly suggests that initially the ADH is involved and that the methanol methyltransferase is operating later at lower methanol concentrations.

### Role of hydrogenases in the alcohol metabolism

Genes coding for four hydrogenases were described to be present in the genome of *D. kuznetsovii* (Desku_0995, 2307–2309, 2934, 2995–2997). All four are cytoplasmic FeFe hydrogenases. Two were suggested to be confurcating (Desku_2307–2309; 2995–2997) due to their similarity to the bifurcating/confurcating hydrogenases of *Pelotomaculum thermopropionicum*. The two possible confurcating

---

Fig. 4 Neighbor-joining tree based on ADH amino acid sequences. The sequences were obtained from a BLASTp analysis, using ADHs of *D. kuznetsovii* as query sequences. ADHs of *D. kuznetsovii* are printed in bold and an arrow points at the methanol-oxidizing ADH. Closed circles represent bootstrap values of 75% or higher. Scale bar represents 10% sequence difference.

Fig. 5 Stable carbon isotope fractionation analysis of *D. kuznetsovii*. a Percentage of methanol degraded in time. b SCIF analysis data, presented as the delta $^{13}$C fractionation values of methanol set out against the methanol degradation. Open symbols are controls without bacteria.
hydrogenases were synthesized during growth of *D. kuznetsovii* with different substrates (Fig. 1), while the other two predicted hydrogenases were not identified in the proteome data. One of the confurcating hydrogenases (*Desku_2307–2309*) showed increased abundance during growth with methanol, whereas the other hydrogenase (*Desku_2995–2997*) was more abundant when *D. kuznetsovii* was grown with lactate or ethanol.

As the ADH was shown to reduce NAD+34, the NADH and reduced ferredoxin formed by the ADH and the aldehyde ferredoxin oxidoreductase, respectively, could be used by the confurcating hydrogenase to form hydrogen. Subsequently, hydrogen could be used to reduce sulfate as proposed in a hydrogen-cycling model for sulfate reducers44. The abundance of the two hydrogenases was associated with the hydrogen levels that could be measured in the cultures. When grown with ethanol the hydrogen levels reached values of around 3000 ppm, while with methanol the hydrogen level was substantially lower (highest value about 550 ppm).

Environmental implications. The presence of two methanol-degradation pathways may be beneficial for *D. kuznetsovii* in its deep-subsurface habitat where it has to compete with other methylo trophic anaerobes. Generally, methanogens and acetogens grow faster with methanol than sulfate reducers, but their growth is hampered by cobalt limitation35,36,45. Methanogens appear to compete better for cobalt during cobalt-limiting conditions36, while acetogens outcompete methanogens when the concentrations of methanol and cobalt are high35,36. Mixed culture experiments of the acetogen *Moorella thermautotropha* and *D. kuznetsovii* at methanol-limiting conditions showed that *D. kuznetsovii* has a higher affinity for methanol46. Owing to the two methanol-degrading pathways *D. kuznetsovii* can successfully compete with both methanogens and acetogens. During cobalt-limiting conditions, *D. kuznetsovii* can compete with methanogens because of the cobalt-independent pathway; and when cobalt is not limiting, but methanol concentrations are low, *D. kuznetsovii* can compete with acetogens by virtue of its methanol methyltransferase pathway.

Methanol is a common substrate in both aerobic and anaerobic environments. To analyze methanol utilizers in the environment molecular tools are required. Kolb and Stacheter addressed this issue47. To get a better understanding of the global methanol conversion, they discussed the need for suitable gene targets to analyze methanol-utilizing microorganisms. Moreover, they identified five potential gene markers for aerobes and one for strict anaerobes, the *mtaC* gene47. However, the *mtaB* gene is a better alternative as a target to develop gene-based detection of strict anaerobic methanol utilizers in the environment, because the *mtaB* codes for the methanol specific subunit of the methyltransferase. Furthermore, the MtaC has high similarity with the cobalamin binding subunits of the tri-, di-, and mono-methylamine methyltransferases. In addition to *mtaB*, another gene marker needs to be developed to target methanol-utilizing microorganisms that employ the MDH pathway as found in *D. kuznetsovii*. However, the methanol-oxidizing ADH of *D. kuznetsovii* clusters together with ADHs of species that cannot grow with methanol (Fig. 4). More methanol-degrading SRB should be investigated to assess if the use of a methanol-oxidizing alcohol dehydrogenase is more common among sulfate reducers. Moreover, finding more of these proteins will help establishing the difference with only ethanol-oxidizing ADHs and will lead to a suitable gene marker.

New hypothetical energy-conserving formate dehydrogenase complex. Growth of *D. kuznetsovii* on lactate led to increased abundances of lactate transporter and lactate dehydrogenase (*Desku_2393–2395*), pyruvate formate lyase (*Desku_2520*) and likely a formate dehydrogenase complex (*Desku_0187–0192*). The use of a pyruvate formate lyase instead of ferredoxin-oxoacid (pyruvate ferredoxin oxidoreductase (*Deku_0030–0033*)), which is not more abundant when grown with lactate, might be beneficial from the energetic point of view. Research with *D. vulgaris* indicated that intracellular cycling of formate formed by pyruvate formate lyase might contribute to energy conservation48. Formate conversion to hydrogen and carbon dioxide indeed is coupled to energy conservation and growth of *Desulfovibrio*, even in the absence of sulfate49,50. Interestingly, the presumed formate dehydrogenase complex of *D. kuznetsovii* does not have much similarity with any of the formate dehydrogenases of *D. vulgaris*. The formate dehydrogenase complexes (*Desku_0187–0192* and *Desku_2987–2991*) need to be studied further. The abundant protein complex when grown with lactate, *Desku_0187–0192*, consists of five subunits. *Desku_0187* and 0188 are annotated as a glutamate synthase and a FAD dependent oxidoreductase, respectively. Both protein sequences contain several pyridine-nucleotide-disulfide oxidoreductase domains, which indicates that these subunits are the catalytic subunits of the protein complex. The annotated glutamate synthase (*Desku_0187*) has about 62% similarity with a FAD nucleotide disulfide oxidoreductase of *Desulfitomaculum ruminis*. The *Desku_0189* is annotated as a methylviologen-dependent hydrogenase. The protein annotated as a formate dehydrogenase (*Desku_0190*) contains a 4Fe–4S cluster, but lacks the characteristic catalytic domain of other formate dehydrogenases. Therefore, we hypothesize that this enzyme complex concerns a novel type of energy-conserving formate dehydrogenase complex. The protein sequence of *Desku_0192* predicts a Twin-arginine signal peptide cleavage site, but none of the subunit sequences of the complex predicts transmembrane helices. This indicates that the membrane complex is translocated across the membrane. Currently, it is unclear if *Desku_0184–0186* also belong to the enzyme complex. *Desku_0185* is also more abundant when grown with lactate, while *Desku_0184* and *Desku_0186* are not found in the proteome.

Methods

Culture medium and experimental design. *Desulfotomaculum kuznetsovii*10 was grown in bicarbonate buffered medium described by Stams et al.31. To investigate whether a methanol methyltransferase system is involved in methanol conversion, *D. kuznetsovii* was grown with methanol and sulfate in normal medium (i.e., using the trace and vitamin solutions described by Stams et al.31, containing CoCl2 and vitamin B12) and in medium deprived from cobalt (CoCl2) and vitamin B12. Methanol (20 and 5 mM) and sulfate (10 mM) were added from concentrated stock solutions (sterilized by autoclaving). In addition to the four methanol growth conditions (20 mM methanol with and without cobalt and vitamin B12, and 5 mM methanol with and without cobalt and vitamin B12), two other growth conditions were used for a comparative proteomics analysis. Those growth conditions were: lactate (20 mM) with sulfate (10 mM) and ethanol (20 mM) with sulfate (10 mM) (both in medium containing cobalt and vitamin B12).

Cultivation of *D. kuznetsovii* was performed at pH 7 and 60 °C in 117 mL glass serum bottles with butyl rubber stoppers and aluminum crimp seals. The bottles contained 50 mL basal medium and a gas phase of 1.7 bar N2/CO2 (80%/20%, vol/vol). In initial growth experiments and the stable isotope fractionation experiment the inoculum size was 1% (vol/vol) and cultures were transferred at least five times to ensure full adaptation to the growth substrate. For proteomics, cultures were transferred at least ten times. Assays for proteomics were performed in triplicate or quadruplicate.

Growth was recorded by monitoring the optical density at 600 nm (U-1300 spectrophotometer Hitachi), by gas chromatographic determination of the methanol concentration (using a GC-2010, Shimadzu, equipped with a Sil 5 CB column), and by measuring sulfate concentrations using ion chromatography (an ICS2100 system, Thermo Scientific, equipped with an AS19 column). Sulfide was measured photometrically with the methylene blue method52. Hydrogen in bottles’ headspace was monitored by gas chromatography (using a Compact GC40, Global Analyser Solutions, equipped with Carbosieve 1010 column (Supelco, 3 m × 0.32 mm).
Prior to the stable isotope fractionation analysis calcium chloride was added to precipitate the carbonate from the medium and the medium was centrifuged (Minispin®, Eppendorf, Hamburg, Germany) for 5 min at 12,000×g and room temperature to remove the carbonate and iron precipitates. High performance liquid chromatography coupled via LC-Isolink interface to a stable isotope ratio mass spectrometer MAT 253 (Thermo Fisher Scientific, Bremen, Germany) was used to determine the carbon isotope ratios of methanol following the principle of a wet chemical oxidation as described before.

The HPLC system was further equipped with a HT PAL autosampler (CTC Analytics, Zwingen, Switzerland), a Surveyor MS Pump Plus (Thermo Fisher Scientific, Bremen, Germany), and a HT HPLC 200 column oven (StM, Oberhausen, Germany). Sample aliquots (10 μL) were separated on an Atlantis T3 column (150 μm × 3 mm, 3 μm inner diameter; Waters, Eschborn, Germany), equipped with a 10 μm × 2.1 mm pre-column (Waters, Eschborn, Germany) at 40 °C using Milli-Q water with a flow rate of 100 μL/min as eluent. The wet chemical oxidation of methanol was achieved by online mixing with ortho-phosphoric acid (0.75 M) and sodium peroxide-sulfate (200 μL) prior to entering the oxidation reactor. The reagents were pumped separately by two pumps with flow rate of 50 μL/min each. The temperature of the reactor was maintained at 99.9 °C. All samples were measured in triplicate, and the typical uncertainty of analysis was < 0.4‰. Enrichment factors and standard deviations were calculated as described in Jekel et al. The error of the enrichment factor is given as 95% confidence interval (CI), determined using a regression analysis as described by Ehlert et al.

Data availability. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the data set identifier PXD006899. All other relevant data are available in this article and its Supplementary Information files, or from the corresponding author upon request.

Received: 24 October 2016 Accepted: 6 December 2017
Published online: 16 January 2018

References

1. Gniwe, C. et al. Relevance of deep-subsurface microbiology for underground storage and geothermal energy production. Adv. Biochem. Eng. Biotechnol. 142, 95–121 (2014).
2. Moser, D. P. et al. Desulfitomaculum and Methanobactera spp. dominate a 4- to 5-kilometer-deep fault. Appl. Environ. Microbiol. 71, 8773–8875 (2005).
3. Basso, O., Lascourreges, J. F., Le Borgne, F., Le Goff, C. & Magot, M. Characterization by culture and molecular analysis of the microbial diversity of a deep subsurface gas storage aquifer. Res. Microbiol. 160, 107–116 (2009).
4. Aüllo, T., Ranchou-Peyruse, A., Ollivier, B. & Magot, M. Desulfitomaculum spp. and related gram-positive sulfate-reducing bacteria in deep subsurface environments. Front. Microbiol. 4, 362 (2013).
5. Puente-Sánchez, F. et al. Deep subsurface sulfate reduction and methanogenesis in the Iberian Pyrite Belt revealed through geochemistry and molecular biomarkers. Geobiology 12, 34–47 (2014).
6. Kuever, J. et al. Genome analysis of Desulfitomaculum gibsoniae strain Groll(T) a highly versatile Gram-positive sulfate-reducing bacterium. Stand. Genom. Sci. 9, 821–839 (2014).
7. Visser, M. et al. Genome analysis of Desulfitomaculum kuznetsovii strain 17(T) reveals a physiological similarity with Pelotomasculum thermopropionicum strain S(T). Stand. Genom. Sci. 1, 69–87 (2013).
8. O’Sullivan, L. A. et al. Survival of Desulfitomaculum spores from estuarine sediments after serial autoclaving and high-temperature exposure. ISME J. 9, 922–933 (2015).
9. Dallavacca, E., Visser, M., Stams, A. J. M. & Bernier-Latmani, R. Investigation of sporation in the Desulfitomaculum genus: a genomic comparison with the genera Bacillus and Clostridium. Environ. Microbiol. Rep. 6, 756–776 (2014).
10. Nazina, T. N., Ivanova, A. E., Kanchaveli, L. P. & Rozanova, E. P. A new spore forming thermophilic methyloptic sulfate-reducing bacterium Desulfitomaculum kuznetsovii sp. nov. Nov. Microbiol. 57, 823–827 (1988).
11. Yanagawa, K. et al. Biogeochecmical cycle of methanol in anoxic deep-sea sediments. Microbes Environ. 31, 190–193 (2016).
12. Schink, B. & Zekos, J. G. Microbial methanol formation: a major end product of pectin metabolism. Curr. Microbiol. 4, 387–389 (1980).
13. Voglesonger, K. M., Holloway, J. R., Dunn, E. E. Dalla-Betta, P. J. & O’Day, P. A. Experimental abiotic synthesis of methanol in seafloor hydrothermal systems during diving events. Chem. Geol. 180, 129–139 (2001).
14. Anthony, C. & Williams, P. The structure and mechanism of methanol dehydrogenase. Biochin. Biophys. Acta 1647, 18–23 (2003).
15. Hектор, H. J., Kroosterman, H. & Dijkhuizen, L. Nicotinoprotein methanol dehydrogenase enzymes in Gram-positive methylothetic bacteria. *J. Mol. Biol. Enzym.* 38, 103–109 (2000).

16. Vu, H. N. et al. Lanthanide-dependent regulation of methanol oxidation systems in *Methylbacterium extorquens* AM1 and their contribution to methanol growth. *J. Bacteriol.* 198, 1250–1259 (2016).

17. Harms, U. & Thauer, R. K. Methylcobalamin: coenzyme M methyltransferase isoenzymes MtaA and MtbA from *Methanosarcina* bacteria. Cloning, sequencing and differential transcription of the encoding genes, and functional overexpression of the mtaA gene in *Excherichia coli*. *Eur. J. Biochem.* 235, 653–659 (1996).

18. Sauer, K., Harms, U. & Thauer, R. K. Methanol:coenzyme M methyltransferase from *Methanosarcina barkeri*. Purification, properties and encoding genes of the corrinoid protein MT1. *Eur. J. Biochem.* 151, 670–677 (1987).

19. van der Meijden, P. et al. Methyltransferases involved in methanol conversion by *Methanosarcina* bacteria. *Arch. Microbiol.* 134, 238–242 (1983).

20. van der Meijden, P., te Brommelstroet, B. W., Poirot, C. M., van der Drift, C. & Vogels, G. D. Purification and properties of methanol-5-hydroxybenzimidazolocobamide methyltransferase from *Methanosarcina barkeri*. *J. Bacteriol.* 160, 629–635 (1984).

21. Das, A. et al. Characterization of a corrinoid protein involved in the C1 metabolism of strict anaerobic bacterium *Moorella thermoacetica*. *Proteins* 67, 167–176 (2007).

22. Pierse, E. et al. The complete genome sequence of *Moorella thermoacetica* (f. *Chromatium*). *Environ. Microbiol.* 10, 2550–2573 (2008).

23. Stupperich, E. & Konle, R. Corrinoid-dependent methyl transfer reactions are involved in methanol conversion by *Methanosarcina barkeri*. *Arch. Microbiol.* 134, 238–242 (1983).

24. Klemps, R., Cypionka, H., Widdel, F. & Pfennig, N. Growth with hydrogen, and hydrogen stable isotope fractionation associated with the anaerobic degradation of methanol. *Desulfobacterium catecholicum*. *J. Mol. Microbiol. Biotechnol.* 143, 143–147 (1998).

25. Liu, Y. T. et al. Description of two new thermophilic *Desulfotomaculum* strains with methanol and sulfoxide utilisation in the *Desulfotomaculum* sp. *Int. J. Syst. Evol. Microbiol.* 54, 1223–1229 (2004).

26. Schnell, S., Bak, F. & Pfennig, N. Anaerobic degradation of aniline and dihydroxycobenzene by newly isolated sulfate-reducing bacteria and description of *Desulfofaricium anilinum*. *Arch. Microbiol.* 152, 556–563 (1989).

27. Nanninga, H. J. & Gottschal, J. C. Properties of *Desulfovibrio carboxidus* sp. nov. and other sulfate-reducing bacteria isolated from an anaerobic purification plant. *Appl. Environ. Microbiol.* 53, 802–809 (1987).

28. Qatib, A. I., Niviere, V. & Garcia, J. L. *Desulfovibrio alcoholovorans* sp. nov, a sulfate-reducing bacterium able to grow on glycerol, 1,2-propanediol and 1,3-propanediol. *Arch. Microbiol.* 155, 143–148 (1991).

29. Balk, M. et al. Methanol utilizing *Desulfotomaculum* species utilizes hydrogen in a methanol-fed sulfate-reducing bioreactor. *Appl. Microbiol. Biotechnol.* 73, 1203–1211 (2007).

30. Fardeau, M. et al. Isolation and characterization of a thermostable sulfate-reducing bacterium *Desulfotomaculum thermosavoryanus* sp. nov. *Int. J. System. Bacteriol.* 45, 218–221 (1995).

31. Gilevska, T., Gehre, M. & Richnow, H. H. A liquid chromatography–mass spectrometry method for the detection of 1-halo and 1,1-dihalo alkanes in the environment. *J. Chromatogr. A* 919, 213–224 (2001).

32. Liao, X. et al. Description of two new thermophilic *Desulfotomaculum* spp., *Desulfotomaculum putei* sp. nov, from a deep terrestrial subsurface, and *Desulfotomaculum luciae* sp. nov, from a hot spring. *Int. J. System. Bacteriol.* 47, 615–621 (1997).

33. Tebo, B. M. & Obraztsova, A. Y. Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mo(VI), and Fe(III) as electron acceptors. *FEBS Microbiol. Lett.* 162, 193–198 (1998).

34. Gilevska, T. P. Methanol dissimilation in *Desulfotomaculum* kuznetsovii. Ch 3: Thermophilic methanolic utilization by sulfate reducing bacteria PhD dissertation, 55–61 (2002).

35. Florence, L., Field, J. A. & Lettinga, G. Importance of cobalt for individual trophic groups in an anaerobic methanol-degrading consortium. *Appl. Environ. Microbiol.* 60, 227–234 (2002).

36. Florence, L., Jenicek, P., Field, J. A. & Lettinga, G. Effect of cobalt on the anaerobic degradation of methanol. *J. Ferment. Bioeng.* 75, 368–374 (1993).

37. Penger, I., Conrad, R. & Blaser, M. Stable carbon isotope fractionation by methylthropic methanogenic archaea. *Appl. Environ. Microbiol.* 78, 7596–7602 (2012).

38. Kryzczyk, J. A., Keenezly, W. R., DeNiro, M. J. & Zeikus, J. G. Stable carbon isotope fractionation by *Methanosarcina barkeri* during methanogenesis from acetate, methanol, or carbon dioxide-hydrogen. *Appl. Environ. Microbiol.* 53, 1997–1999 (1987).

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

40. Paul, P. L., Jiang, B., Cysneiros, D., Stams, A. J. M. & Lettinga, G. Effect of cobalt on the anaerobic thermophilic conversion of methanol. *Biotechnol. Biofuels* 4, 434–441 (2011).

41. Stupperich, E. & Konle, R. Methanol:coenzyme M methyltransferase from *Methanosarcina barkeri* sub-stitution of the corrinoid harbouring subunit MtaC by free cob(II)alamin. *Eur. J. Biochem.* 261, 674–681 (1999).

42. Stupperich, E. & Konle, R. Corrinoid-dependent methyl transfer reactions are involved in methanol and 3,4-dimethoxynitrate metabolism by *Sporomusa ovata*. *Appl. Environ. Microbiol.* 59, 3110–3116 (1993).

43. Hagemeyer, C. H., Kruger, M., Thauer, R. K., Warkentin, E. & Ermier, U. Insight into the mechanism of biological methanol activation based on the crystal structure of the methanol-cobalamin methyltransferase complex. *Proc. Natl Acad. Sci. USA* 103, 18917–18922 (2006).

44. Sauer, K. & Thauer, R. K. Methanol:coenzyme M methyltransferase from *Methanosarcina barkeri* substitution of the corrinoid harbouring subunit MtaC by free cob(II)alamin. *Eur. J. Biochem.* 261, 674–681 (1999).

45. Perdigon, J., Conrad, R. & Blaser, M. Stable carbon isotope fractionation by methylthropic methanogenic archaea. *Appl. Environ. Microbiol.* 78, 7596–7602 (2012).

46. Vezzani, J.A. et al. Update of the PRIDE database and related tools. *Nucl. Acids Res.* 44, D447–D456 (2016).

Acknowledgements

Research was funded by grants of the Division of Chemical Sciences (CW-TOP 700.55.343) and Earth and Life Sciences (ALW 819.02.014) of The Netherlands Organisation for Scientific Research (NWO), the European Research Council (ERC grant 338309), and the devastation grant 024.002.002 of the Netherlands Ministry of Education, Culture and Science.

Author contributions

D.Z.S., M.V. and A.H.V. performed physiological and proteomics experiments and analyzed the data. S.B., M.M.P., M.W.H.P., D.J.E.M.V. did peptide analyses and analyzed proteomics data. C.V., S.F. and S.K. performed stable isotope fractionation analysis and

8 | NATURE COMMUNICATIONS | DOI: 10.1038/s41467-017-02518-9 | www.nature.com/naturecommunications
contributed with the data analysis. M.V., D.Z.S. and A.J.M.S. designed the experiments and wrote the manuscript. All authors agreed with the final version of the manuscript.

**Additional information**

Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-017-02518-9.

**Competing interests:** The authors declare no competing financial interests.

**Reprints and permission** information is available online at http://npg.nature.com/reprintsandpermissions/

**Publisher’s note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.