How sulphate-reducing microorganisms cope with stress: Lessons from systems biology

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Abstract | Sulphate-reducing microorganisms (SRMs) are a phylogenetically diverse group of anaerobes encompassing distinct physiologies with a broad ecological distribution. As SRMs have important roles in the biogeochemical cycling of carbon, nitrogen, sulphur and various metals, an understanding of how these organisms respond to environmental stresses is of fundamental and practical importance. In this Review, we highlight recent applications of systems biology tools in studying the stress responses of SRMs, particularly *Desulfovibrio* spp., at the cell, population, community and ecosystem levels. The syntrophic lifestyle of SRMs is also discussed, with a focus on system-level analyses of adaptive mechanisms. Such information is important for understanding the microbiology of the global sulphur cycle and for developing biotechnological applications of SRMs for environmental remediation, energy production, biocorrosion control, wastewater treatment and mineral recovery.

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The ability of microorganisms to sense and respond rapidly to adverse changes in the environment is crucial to their survival. Intensive studies of stress responses have focused primarily on *Escherichia coli*[1], *Bacillus subtilis*[2–4] and *Saccharomyces cerevisiae*[5], and have provided insights into the physiology of these organisms and their regulation of gene expression in response to environmental changes. However, without a more thorough sampling of physiologically and phylogenetically diverse microbial species, it is impossible to know which aspects of these stress response mechanisms, if any, are universal. A comparative analysis of >200 sequenced microbial genomes has indicated that many signalling and regulatory systems are not found in the key model microorganisms[6], indicating the need to characterize other organisms. Until recently, however, such data have been scarce because of a lack of appropriate genetic, biochemical and genomic tools.

One organism for which such tools have been recently developed is the sulphate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough (hereafter referred to as *D. vulgaris* H.). Originally isolated from clay soil near Hildenborough, Kent, UK, *D. vulgaris* H. is an anaerobic deltaproteobacterium with an evolutionary history and a physiology that is distinct from the model organisms mentioned above. This bacterium is traditionally grouped with other sulphate-reducing microorganisms (SRMs), a group that includes diverse bacterial and archaeal lineages[7,8]. SRMs are characterized by the ability to carry out dissimilatory sulphate reduction (that is, energy generation by coupling the oxidation of organic compounds or H_2 to the reduction of sulphate (SO_4^{2-}) to sulphide (S^{2-}) and other sulphur-containing compounds[9]), which directly links these organisms to the natural cycling of both carbon and sulphur (FIG. 1). The activities of SRMs shape the global sulphur cycle and, given that sulphur is one of the most abundant elements on Earth, represent important linkages to the global cycling of other elements such as carbon[7] (FIG. 1). *D. vulgaris* H. can be grown and manipulated in the laboratory with ease; thus, this strain has been established as a useful model for the study of SRMs and as a representative of the broadly distributed *Desulfovibrio* genus, which is found in a variety of habitats[7,10–15].

Much of the past interest in SRMs has been focused on their involvement in biocorrosion of ferrous metal installations in the petroleum industry [16,17] and of concrete structures in wastewater collection systems[18,19]. More recent studies[20] have documented the potential of SRMs in the bioremediation of toxic heavy metals and

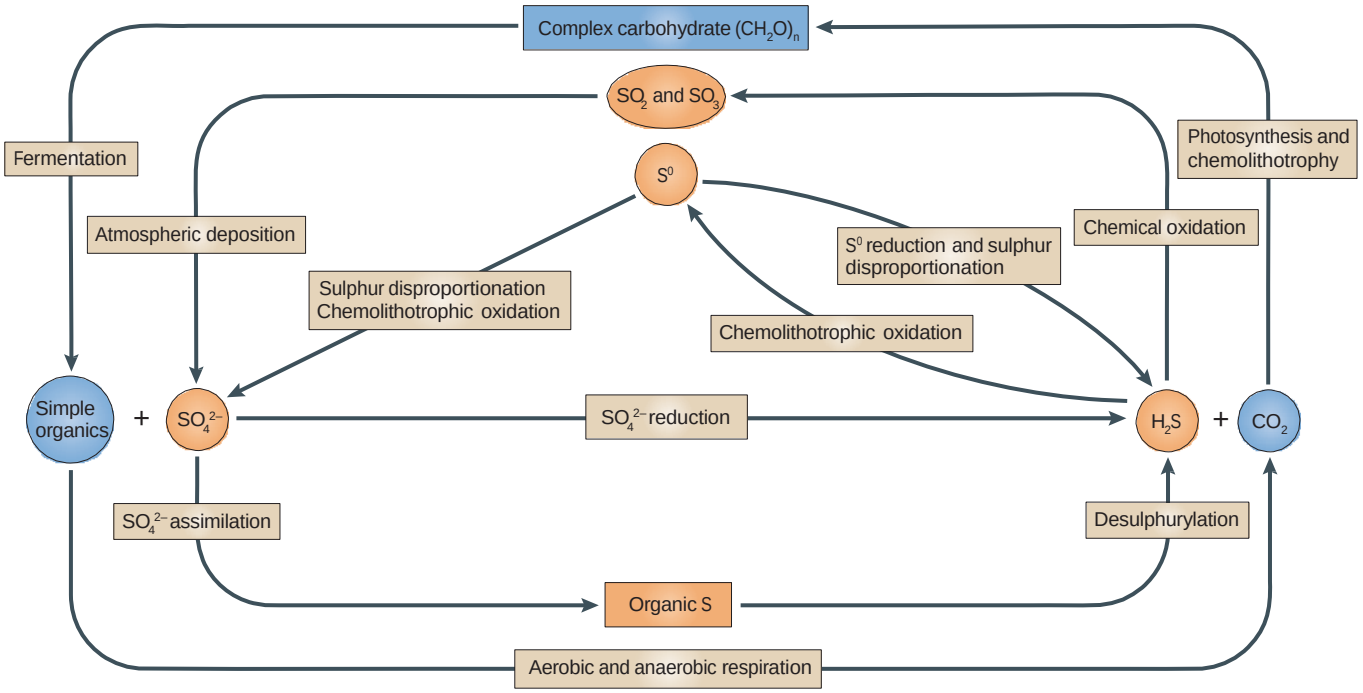


Figure 1 | Sulphate-reducing microorganisms and the carbon and sulphur cycles. Sulphate-reducing microorganisms (SRMs) use sulphate (SO_4^{2-}) as the terminal electron acceptor during the degradation of simple organic matter. This reduction of SO_4^{2-} produces hydrogen sulphide (H_2S) and carbon dioxide (CO_2). Thus, SRMs play important parts in the natural cycling of both sulphur and carbon (orange and blue pathways, respectively). As a product of SO_4^{2-} reduction, H_2S can be subsequently oxidized by chemolithotrophic organisms to elemental sulphur (S^0) and further to SO_4^{2-}. Sulphate can also be derived from atmospheric deposition of sulphur oxides that are formed from the chemical oxidation of H_2S. Subsequently, SO_4^{2-} can be again reduced by SRMs to H_2S, or taken up as a required nutrient by many organisms to form organic sulphur. Desulphurylation of organic sulphur during the decomposition of dead organisms releases the sulphur again as H_2S. Other biotransformations in the sulphur cycle include the reduction of S to H_2S and sulphur disproportionation, in which S^0 is converted into both H_2S and SO_4^{2-}. The role of SRMs in carbon cycling is linked to the utilization of simple organics, such as organic acids, as the electron donors in SO_4^{2-} reduction. CO_2, one of the end products of SO_4^{2-} reduction, enters the global carbon cycle and can be fixed into complex carbohydrates by photosynthesis or chemolithotrophy. These complex carbohydrates can be further fermented into simple organics, which are then used for SO_4^{2-} reduction or other modes of metabolism.

Stress
A deviation from optimal growth conditions that leads to a reduced growth rate or cellular damage as a result of environmental or internal changes.

Adaptations
Genetically encoded traits that enhance the fitness of their bearers.

Functional genomics
Large-scale genomic studies that use functional measurements such as changes in the levels of mRNAs, proteins and metabolites, combined with statistical analyses, mathematical modelling and computational analysis of the results, to gain knowledge of cell physiology.

Syntrophic
Pertaining to a type of mutualism in which two or more species cooperate to complete a single energy-yielding reaction from which neither species alone can gain energy.

Metagenomic
Pertaining to the study of microbial community genomes directly from environmental samples using high-throughput sequencing and associated genomics technologies.

radionuclides such as chromium and uranium[7,8,21,22]. Several recent reviews provide an excellent overview of the progress that has been made in our understanding of the biochemistry, molecular biology, physiology and ecology of SRMs, as well as their biotechnological applications[7,8,23]. Here, we attempt to integrate our understanding of the responses and the adaptations of SRMs to environmental stresses at the cell, population, community and ecosystem levels using a variety of integrated systems biology approaches (BOX 1). First, we highlight several studies that used comparative genomics as well as integrated functional genomics to investigate the responses of *D. vulgaris* H. (as a model SRM) to various environmental stresses. Then, we provide a brief description of the adaptive responses of this strain during its syntrophic growth with other microorganisms. Finally, we discuss recent metagenomic studies of the responses of SRMs to environmental stresses, within the context of environmental remediation.

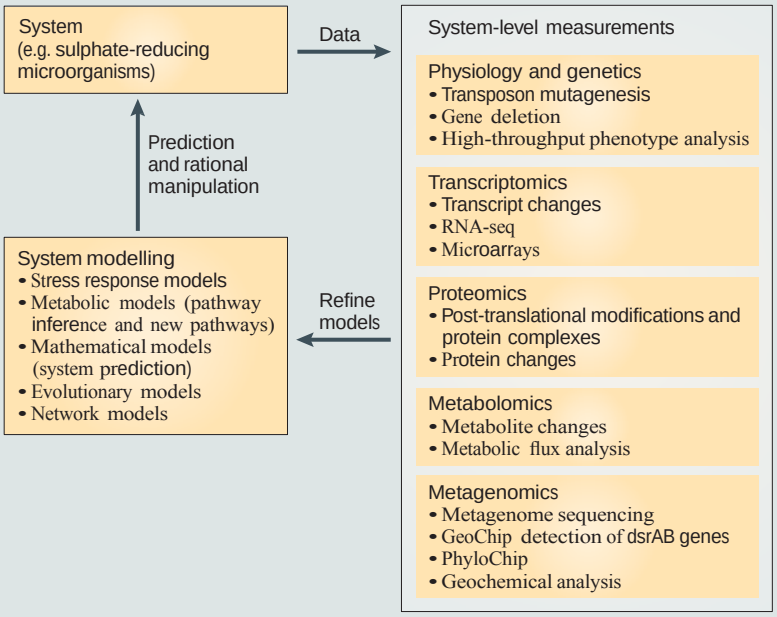
Comparative genomics of SRMs

The past 10 years have provided a wealth of genomic information for various SRM species. Altogether, a total of 23 genomes have been sequenced from four phylogenetically distinct lineages of SRMs (FIG. 2; TABLE 1): the bacterial class Deltaproteobacteria (the most highly represented lineage among SRMs), phylum Firmicutes and phylum Nitrospirae, and the archaeal phylum Euryarchaeota. These microorganisms were isolated from a variety of habitats, including soil, fresh water, marine sediments, animal gastrointestinal tracts and metal corrosion sites[24–31]. Sequences are not yet available for SRMs that represent other major lineages, such as the crenarchaeotal genera *Caldivirga* and *Thermocladium*, or for the recently isolated *Thermodesulfobium narugense*, a species of uncertain phylogenetic affiliation within the Bacteria. Below, we discuss comparative genomic analyses that relate to energy metabolism and signal transduction, two pathways that are central to the sensing of and acclimation to stresses.

Hydrogen-cycling models. A long-standing puzzle posed by the energetics of sulphate reduction is how SRMs can generate sufficient energy to support growth, given that sulphate must be activated by hydrolysis of the equivalent of two ATP molecules[32]. Unlike most terminal electron acceptors used under anaerobic conditions, which

Box 1 | Systems biology for studying sulphate-reducing microorganisms

The term 'systems biology' is widely used in the scientific community and has been contrived to attract attention, but its exact meaning is poorly defined. Here, we refer to systems biology as a field in biology that aims to use high-throughput genomic, computational and mathematical tools to understand, predict and/or control the structure, functions, interactions, dynamics and evolution of biological systems across different organizational levels, such as macromolecules, cells, individuals, populations, communities and ecosystems[144,146]. A variety of 'omics' tools, targeting biological systems at various scales, are used in combination with conventional genetic and biochemical approaches to obtain system-level measurements for subsequent modelling and simulation of the system under study (see the figure). For instance, microbial populations can be phenotypically characterized in terms of their biochemistry, physiology and ecology and then analysed using high-throughput 'omics' tools, such as those provided by transcriptomics (for example, microarrays, RNA-sequencing (RNA-seq) or whole-transcriptome shotgun sequencing), proteomics (for example, mass spectrometry to identify proteins, protein complexes and post-translational modifications)[37,48,51,147,148] and metabolomics (for example, metabolite profiling and analysis of metabolite fluxes)[37,38,55,149]. At the community scale, high-throughput metagenomic technologies, such as large-scale genome sequencing[97], GeoChip[15,109] and PhyloChip[108], can be applied to monitor the dynamics of microbial communities. The data obtained through the above approaches can then be integrated by system modelling and simulation (for example, by pathway inference and the discovery of new pathways[149]; by the development of models for cellular stress responses[37–40,48,51,53–55], for the evolutionary trajectories of genes[85], pathways, cells, populations and communities[97], and for cellular and community networks[51,150]; and possibly by mathematical modelling, simulation and prediction of stress responses across different organizational levels such as cells, populations and communities).



dsrAB, dissimilatory sulphite reductase subunit-α and subunit-β genes.

are reduced externally or in the periplasm, sulphate is reduced in the cytoplasm by soluble reductases and must first be activated by two ATP equivalents before reduction can occur. Although the partial oxidation of organic acids to acetate can provide the two ATP molecules that are required for sulphate activation, alternative means of ATP generation are needed to generate sufficient energy for growth.

The observation of a transient burst of H_2 in batch cultures of *Desulfovibrio* sp., along with enzyme localization studies, led to an elegant hypothesis to explain the production of energy for growth by SRMs, proposed by Odom and Peck[33] and modified by Voordouw [34]: the hydrogen-cycling model (BOX 2). This model posits that hydrogen equivalents that are generated by the oxidation of organic compounds are converted to H_2 by cytoplasmic hydrogenase complexes. The H_2 is thought to diffuse to the periplasm, where it is metabolized to protons and electrons by periplasmic hydrogenase enzymes[32]. The protons provide a proton-motive force for ATP generation, whereas the electrons are cycled back to the cytoplasm, via the cytochrome c_3 network and various transmembrane complexes, for sulphate reduction and other metabolic processes[35] (BOX 2). Conversely, when H_2 is used as the electron donor, a proton gradient can be established directly by periplasmic oxidation of H_2, although some metabolite cycling is still predicted.

Although the initial genome analysis of *D. vulgaris* H. provided support for the hydrogen-cycling model and identified the putative cytoplasmic hydrogenases involved[24], important mechanistic questions remained. Subsequent analysis of additional SRM genomes found that the enzymes for this system are not absolutely conserved across all species and even show significant diversity within *Desulfovibrio* spp. (see Supplementary information S1 (figure)). All the sequenced SRM genomes encode sulphate reduction enzymes and elements of two electron-transporting enzyme complexes, dissimilatory sulphite reductase (Dsr) and quinone-interacting membrane-bound oxidoreductase (Qmo)[36], which are transmembrane complexes in most strains. Similarly, the quinone reductase complex (Qrc), which acts as a type 1 cytochrome c_3:menaquinone oxidoreductase, is encoded in all the known deltaproteobacterial genomes. By contrast, the cytoplasmic hydrogenases and transmembrane complexes that are putatively involved in hydrogen cycling have a highly variable distribution (see Supplementary information S1 (figure)). For example, the genome of *Desulfovibrio desulfuricans* subsp. *desulfuricans* G20 does not contain the genes encoding the cytoplasmic hydrogenases *Escherichia coli* hydrogenase 3 (Ech) and CO-dependent hydrogenase (Coo) that were identified in *D. vulgaris*, but instead harbours genes for two different putative cytoplasmic hydrogenase complexes. In fact, only some of the >20 sequenced SRM genomes seem to encode Coo and some of the transmembrane complexes, such as the high-molecular-weight cytochrome *c* (Hmc), transmembrane complex (Tmc) and *Rhodobacter* nitrogen fixation NADH–quinone oxidoreductase (Rnf) complexes (see Supplementary information S1 (figure)).

The situation becomes even more complicated when considering SRMs that belong to the Gram-positive phylum Firmicutes. As expected, the Gram-positive *Desulfotomaculum* spp. lack the periplasmic enzymes that are found in Gram-negative SRMs (that is, hydrogenases, formate dehydrogenases and cytochrome c_3), and require a revised model of redox cycling[29]. For example, the Qmo complex in *Desulfotomaculum reducens* is predicted to localize to the cytoplasm and does not seem to be a transmembrane complex, which suggests that it

Signal transduction
A mechanism that converts a mechanical or chemical stimulus into a specific cellular response.

Acclimation
The phenotypic response of a population to a change in environmental conditions.

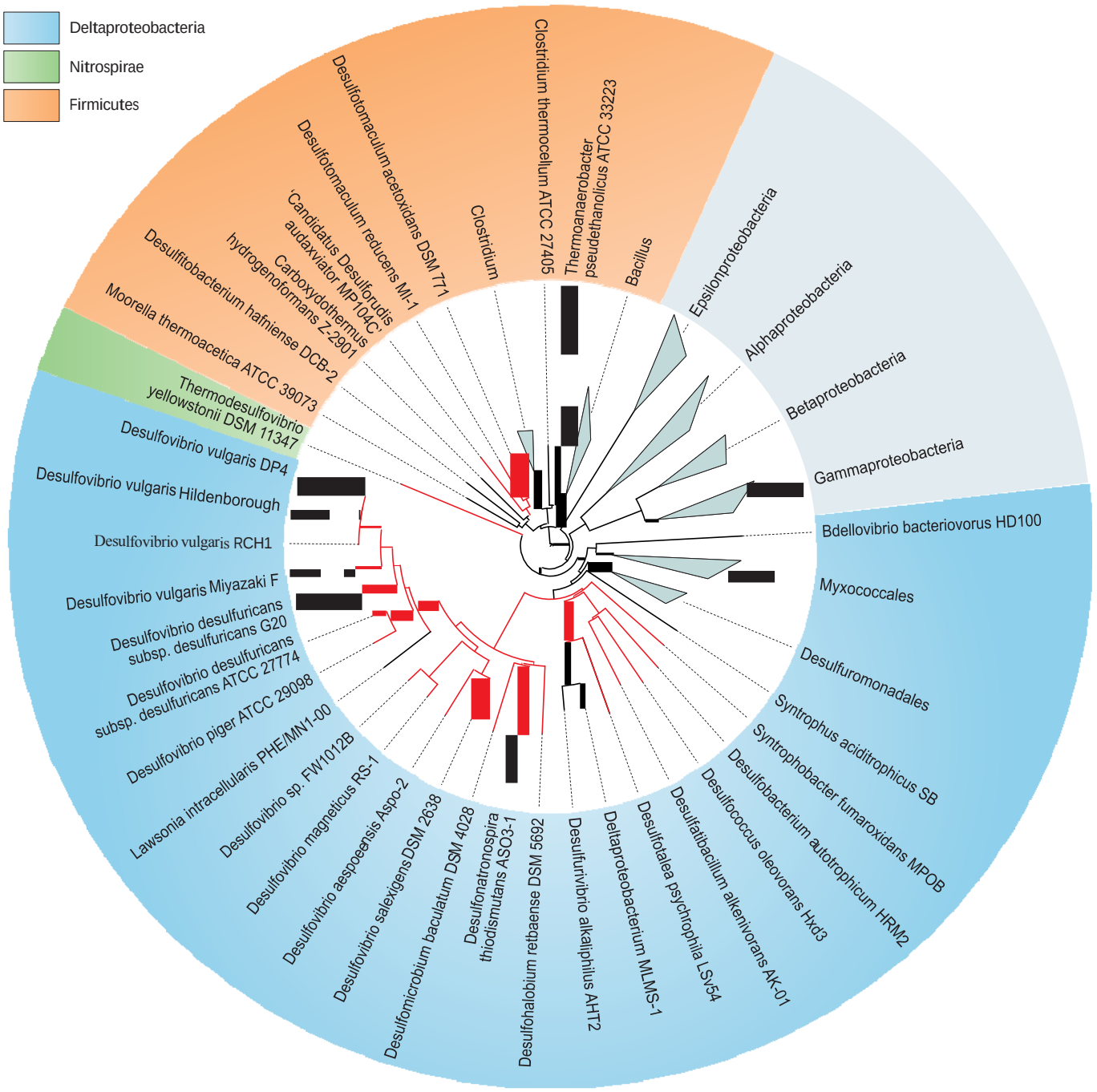


Figure 2 | **Phylogenetic tree of sequenced genomes from sulphate-reducing microorganisms.** A total of 89 genomes were used for tree construction, 23 of which (red branches) are from sulphate-reducing microorganisms (SRMs). Archaeal SRMs are not included in this tree. Genes were identified using AMPHORA[156] and manually annotated to ensure no more than one copy of each reference gene per genome. Single-gene-encoded amino acid alignments were concatenated into a single alignment, and missing peptide sequences were replaced by gaps. The initial tree was constructed using MEGA 4.1 (REF. 157). The evolutionary history was inferred using the neighbour-joining method, and the bootstrap consensus tree was derived from 500 replicates. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. The tree is drawn to scale.

Transcriptomics
The systematic study of a transcriptome (a collection of all of the RNA molecules (mRNA, ribosomal RNA, tRNA and other non-coding RNAs) that are produced in a cell population) using microarrays or sequencing.

accepts electrons from a cytoplasmic source (possibly heterodisulphide reductase) for transport to the adenosine phosphosulphate (APS) reductase [29]. Also, the cytoplasmic pyrophosphatase that drives sulphate reduction in Gram-negative SRMs is thought to be part of a membrane-bound complex in Gram-positive SRMs; it is proposed that this membrane-bound complex might be involved in proton translocation for the establishment of a proton-motive force. Finally, the formate dehydrogenase enzymes in Gram-positive SRMs might be part of

Table 1 | **Sulphate-reducing microorganisms with sequenced genomes**

Organism	Temperature*	Genome size (Mb)	% GC content	Habitat	Characteristics	Accession‡
Domain Archaea, phylum Euryarcheota, class Archaeoglobi						
Archaeoglobus fulgidus DSM 4304	T (83 °C)	2.18	48.58	Geothermal vents	Archaeal model of sulphate-reducing microorganisms	NC_000917
Archaeoglobus profundus DSM 5631	T (82 °C)	1.56	42.00	Geothermal vents	Mixotrophic strain requiring H_2 and acetate for growth	NC_013741
Domain Bacteria, phylum Firmicutes, class Clostridia						
'Candidatus Desulforudis audaxviator MP104C'	T (60 °C)	2.35	60.85	Deep subsurface (2.8 km depth) in a gold mine	Forms single-species communities in the deep subsurface	NC_010424
Desulfotomaculum acetoxidans DSM 771	M (36 °C)	4.55	41.55	Fresh water, ocean or animal waste	Oxidizes acetate to CO_2	NC_013216
Desulfotomaculum reducens MI-1	M (37 °C)	3.61	42.28	Heavy-metal-contaminated sediment	Gram-positive model of sulphate-reducing microorganisms; reduces chromium and uranium	NC_009253
Domain Bacteria, phylum Nitrospirae, class Nitrospira						
Thermodesulfovibrio yellowstonii DSM 11347	T (65 °C)	2.00	34.13	Hot springs	Thermophile	NC_011296
Domain Bacteria, phylum Proteobacteria, class Deltaproteobacteria						
Desulfatibacillum alkenivorans AK-01	M (30 °C)	6.52	54.48	Oil-polluted sediment	Degrades alkenes	NC_011768
Desulfobacterium autotrophicum HRM2	M (30 °C)	5.66	48.76	Ocean	Marine autotroph	NC_012108
Desulfococcus oleovorans Hxd3	M (30 °C)	3.94	56.17	Oil–water mixtures from oil production plants	Degrades alkanes anaerobically	NC_009943
Desulfotalea psychrophila LSv54	P (10 °C)	3.66	46.63	Ocean	Marine psychrophile	NC_006138
Desulfohalobium retbaense DSM 5692	M (37 °C)	2.91	57.33	Hypersaline lake sediment	Halophile	NC_013223
Desulfonatronospira thiodismutans ASO3-1§	M (36 °C)	3.97	51.33	Hypersaline lake sediment	Halophile	ACJN00000000
Desulfomicrobium baculatum DSM 4028	M (36 °C)	3.94	58.65	Manganese ore	Metabolizes H_2 very efficiently	NC_013173
Desulfovibrio aespoeensis Aspo-2§	M (30 °C)	3.57	62.70	Deep groundwater	Lives in a nutrient-poor environment	ADDI00000000
Desulfovibrio desulfuricans subsp. *desulfuricans* G20	M (36 °C)	3.73	57.84	Soil	Has strong bioremediation potential	NC_007519
D. desulfuricans subsp. *desulfuricans* ATCC 27774	M (37 °C)	2.87	58.07	Soil	Reduces nitrate	NC_011883
Desulfovibrio magneticus RS-1	M (36 °C)	5.32	62.67	Soil	Forms magnetosomes	NC_012795
Desulfovibrio piger ATCC 29098§	M (36 °C)	2.83	63.05	Human digestive tract	Commensal of humans	ABXU00000000
Desulfovibrio salexigens DSM 2638	M (37 °C)	4.29	47.09	Marine sediment	Halophile	NC_012881
Desulfovibrio sp. FW1012B§	M (37 °C)	4.18	66.00	Uranium-contaminated groundwater	Isolated from biostimulated, uranium-contaminated groundwater	ADFE00000000
Desulfovibrio fructosovorans JJ§	M (37 °C)	4.67	63.00	Estuarine sediment	Can metabolize fructose	AECZ00000000

Table 1 (cont.) | **Sulphate-reducing microorganisms with sequenced genomes**

Organism	Temperature*	Genome size (Mb)	% GC content	Habitat	Characteristics	Accession‡
Desulfovibrio vulgaris Hildenborough	M (36 °C)	3.77	63.28	Soil	Gram-negative model of sulphate-reducing microorganisms	NC_002937
D. vulgaris DP4	M (36 °C)	3.66	63.16	Freshwater lake sediment	Lacks insertion elements that are present in *D. vulgaris* Hildenborough	NC_008741
D. vulgaris RCH1§	M (36 °C)	3.70	63.00	Chromium-contaminated groundwater	Sequenced for comparative analysis	NA
D. vulgaris 'Miyazaki F'	M (36 °C)	4.04	67.00	Degraded paddy field	Well-characterized hydrogenase	NC_011769
Syntrophobacter fumaroxidans MPOB	M (36 °C)	4.99	60.00	Anaerobic sludge	Syntrophic; degrades propionate	NC_008554

NA, not available. *Temperature characteristics of the species (T, thermophile; M, mesophile; P, psychrophile) followed by optimal growth temperature. ‡For Entrez Genome. §The available sequence data are high-quality drafts (all other genomes mentioned are fully sequenced).

a Na^+-translocating membrane complex, with formate oxidation occurring in the cytoplasm. Pyrophosphatase, the quinone pool and NADH dehydrogenase seem to have a role in the establishment of a proton gradient in *D. reducens*; thus, hydrogen cycling as it is understood in Gram-negative species may not be possible in Gram-positive bacteria.

As expected, few electron transport enzyme complexes are conserved in archaeal SRMs, suggesting that electron transfer for sulphate reduction is radically different between archaea and bacteria. Thus, although the core metabolic machinery for sulphate reduction is conserved in all studied SRMs, there is a substantial variation in the mechanisms of redox cycling and electron flow, and novel mechanisms of energy conservation may remain to be discovered. Indeed, the plethora of redox proteins that are involved in energy metabolism in *D. vulgaris* H. exhibit complex gene expression patterns that are specific to distinct stress conditions, demonstrating the importance of adjustments in energy metabolism pathways as a central strategy in the stress response[37–41]. Even less is known about sulphate reduction and electron transport in sulphate-reducing *Nitrospira* spp. Thus, although hydrogen cycling is an elegant hypothesis to explain energy generation in SRMs, it does not seem to be an absolutely conserved mechanism across all species and may not be present, for example, in Gram-positive bacteria.

Two-component systems. Survival in a fluctuating environment often requires stimulus perception and the subsequent modulation of the expression of relevant genes to optimize metabolism and physiology. In bacteria, these processes are typically mediated by one- or two-component signal transduction systems, the number of which can correlate to the diverse stress responses that are required for the survival of a particular organism[42,43]. One-component signal transduction systems are evolutionarily more ancient and more widely distributed, and display greater diversity in domain composition, than two-component systems[44]. *D. vulgaris* H. has >20 predicted one-component signal transduction proteins; however, little is known about their specific roles.

Although several variations of two-component systems exist, these systems typically include a sensor histidine kinase that either directly or indirectly phosphorylates and consequently activates a downstream response regulator containing the signal output or effector domain[45]. The genome of *D. vulgaris* H. putatively encodes 64 sensor histidine kinases and 72 response regulators, and mechanisms modulated by these proteins are likely to contribute to survival, acclimation and adaptation to the environment. The sensor histidine kinases in *D. vulgaris* H. exhibit an unusual diversity in their domain content and architecture[6].

Response regulators in *D. vulgaris* H. also show considerable diversity, and few have orthologues beyond the sequenced *Desulfovibrio* spp. Furthermore, only 29 of the response regulators from *D. vulgaris* H. contain a DNA-binding output domain, while others contain CheY output domains (which are predicted to act via direct protein–protein interactions in chemotaxis) and domains that regulate cyclic di-GMP levels. Interestingly, 22 of the DNA-binding response regulators fall into the nitrogen regulatory protein C (NtrC) family of response regulators, which are dependent on transcription factor σ^{54} (also known as RpoN). Transcription factor σ^{54} is essential in two deltaproteobacteria: *Myxococcus xanthus* and *Geobacter sulfurreducens.* The unusually large number of σ^{54}-dependent response regulators in *D. vulgaris* H. suggests that they may also have an important role in this organism.

The number of response regulators varies considerably among different SRMs, ranging from 13 in *Desulfovibrio piger* (a human gut isolate) to >70 in most

Proteomics
The large-scale study of proteins, particularly their structures and functions. Mass spectrometry is a popular method for conducting proteomic measurements in a high-throughput manner.

Metabolomics
The systematic study of a metabolome, which is the collection of all the metabolites in a biological cell, tissue, organ or organism.

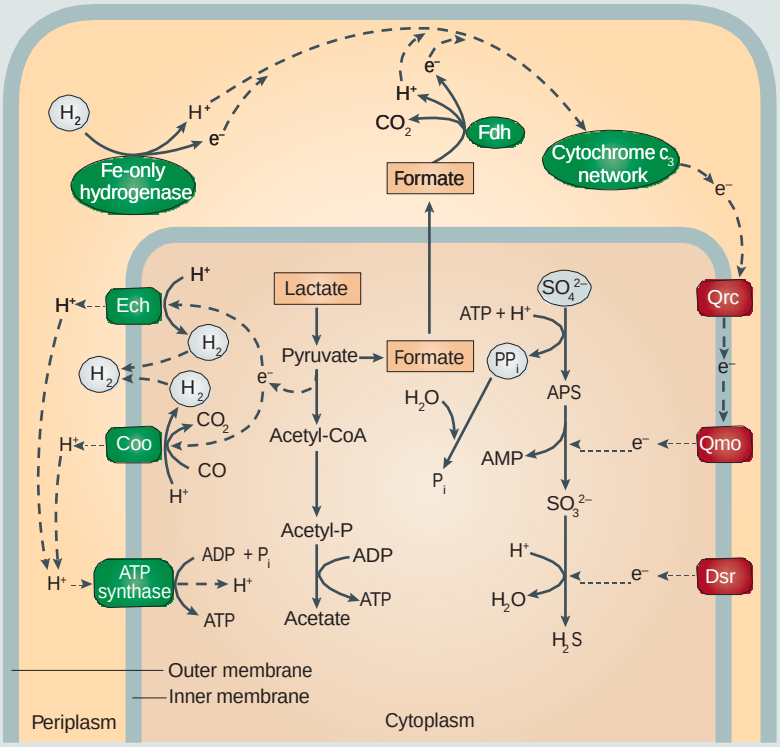
One-component signal transduction systems
Signal-sensing and response systems in which the signal transducer is the direct fusion of an input domain to an output domain in a single protein molecule.

Cyclic di-GMP
A second messenger that is used in signal transduction in a wide variety of bacteria.

Transcription factor σ^{54}
A protein in bacteria that enables binding of RNA polymerase to gene promoters specifically in response to nitrogen limitation.

Box 2 | Hydrogen cycling during dissimilatory sulphate reduction

Early during its growth on lactate and sulphate (SO_4^{2-}), *Desulfovibrio vulgaris* Hildenborough produces a burst of metabolites such as H_2, formate and CO. This observation led to the proposal of the hydrogen-cycling model, which tries to explain the growth of this microorganism despite the energetic constraints that are associated with sulphate reduction[33] (see the figure). According to this model, hydrogen equivalents that are generated by the oxidation of organic compounds (such as lactate) are hypothesized to be cycled to the periplasm via the activities of the cytoplasmic hydrogenases *Escherichia coli* hydrogenase 3 (Ech) and CO-dependent hydrogenase (Coo) (the green pathway in the figure)[34]. In the periplasm, the H_2 is re-oxidized to protons and electrons by the periplasmic hydrogenases, such as the iron-only hydrogenase, and the electrons are passed to the cytochrome c_3 network. From here, electrons are proposed to be transferred to the menaquinone-linked quinone reductase complex (Qrc)[151], then to the quinone-interacting membrane-bound oxidoreductase (Qmo) complex[36] and finally to the adenosine phosphosulphate (APS) reductase for sulphate reduction (the red pathway in the figure). Concurrently, electrons are passed by an unknown mechanism to the dissimilatory sulphite reductase (Dsr) transmembrane complex and then to bisulphite (SO_3^{2-}) reductase. In this way, sufficient electrons are made available for complete reduction of sulphate to hydrogen sulphide (H_2S). The process is made energetically favourable by the activity of inorganic pyrophosphatase, which removes the pyrophosphate (PP_i) that is generated by sulphate activation. Protons that are generated in the periplasm produce the proton-motive force that is necessary for the generation of additional ATP for growth[32]. CO is metabolized in the cytoplasm by CO dehydrogenase, and formate is cycled to the periplasm, where it is metabolized by formate dehydrogenase (Fdh)[34]. Hydrogen cycling is not necessary when H_2 is used as the electron donor, as periplasmic metabolism of H_2 directly establishes the electrochemical gradient that is necessary for ATP synthesis.



P_i, inorganic phosphate.

of the environmental *Desulfovibrio* spp. isolates[42]. The strikingly large numbers of and diversity in the histidine kinases and response regulators in *Desulfovibrio* spp., and the paucity of characterized orthologues, probably reflect the highly fluctuating, multistress environments in which SRMs thrive, and highlight the need to better understand the ecological niches of these organisms. Systematic studies that elucidate the function of these regulatory systems and of the genes that are controlled by them are only now beginning to be carried out and will shed light on a core set of environmental response mechanisms[46,47]. This knowledge is essential if we are to generate predictive models of the stress responses of SRMs to environmental factors, and to develop effective SRM-based biotechnologies.

Functional genomics of stress responses

As *D. vulgaris* H. was the first SRM with a complete genome sequence, it has been used as a model to learn how the ubiquitous SRMs thrive in adverse environmental conditions. In this section, we present an integrated view of the stress responses based on a set of functional genomic analyses of the *D. vulgaris* H. response to various stressors, such as O_2 (REFS 48–50), H_2O_2 (REF. 51), NaCl [37,38], KCl[37], nitrate salts[39], nitrite salts[40,52], heat shock[50,53], starvation[54] and alkaline pH[55].

Energy metabolism. Because the energetics of microbial cells is inherently integrated with their growth, stress responses that typically result in various forms of growth inhibition are expected to be linked to reduced energy metabolism[56]. Indeed, genes that are involved in energy metabolism, such as those encoding the ATP synthase, have lower expression when *D. vulgaris* H. is exposed to heat shock, carbon limitation, nitrate and nitrite salts, air or H_2O_2 (REFS 39,40,48,51,53,54), consistent with the observed repression of energy metabolism as a major stress response in *E. coli*[1,57]. As illustrated in the regulatory cascades that occur in response to nitrite stress[40,52], the reduction in energy production is reflected in the downregulation of the ATP synthase, membrane hydrogenases and the DsrMKJOP transmembrane complex (FIG. 3a), which are key components of the oxidative phosphorylation pathway that is linked to sulphate reduction and hydrogen cycling (BOX 2).

The reduction in energy production creates a dilemma, as substantial quantities of energy and reducing equivalents are typically required by stress-alleviating processes, such as the detoxification of nitrite in the nitrite stress response[40]. This problem is resolved in *D. vulgaris* H. by increasing the flow of reducing equivalents through formate or by, potentially, carrying out 'formate cycling' (FIG. 3b) as an alternative to the classic hydrogen cycling (BOX 2). In fact, all the stress conditions under which energy metabolism is downregulated also result in the increased expression of one or more of the three *fdh* genes, which encode periplasmic formate dehydrogenases (the key enzymes required for formate cycling in *D. vulgaris* H.). However, despite the prevalence of formate as an alternative energy molecule in stress responses, the physiological benefits of using formate over H_2 remain to be elucidated.

Notable exceptions to the stress conditions that result in a reduction in energy metabolism are high salinity (elevated concentrations of KCl and NaCl) and high alkalinity (pH 10), under which expression of the ATP synthase genes is increased. This result could be

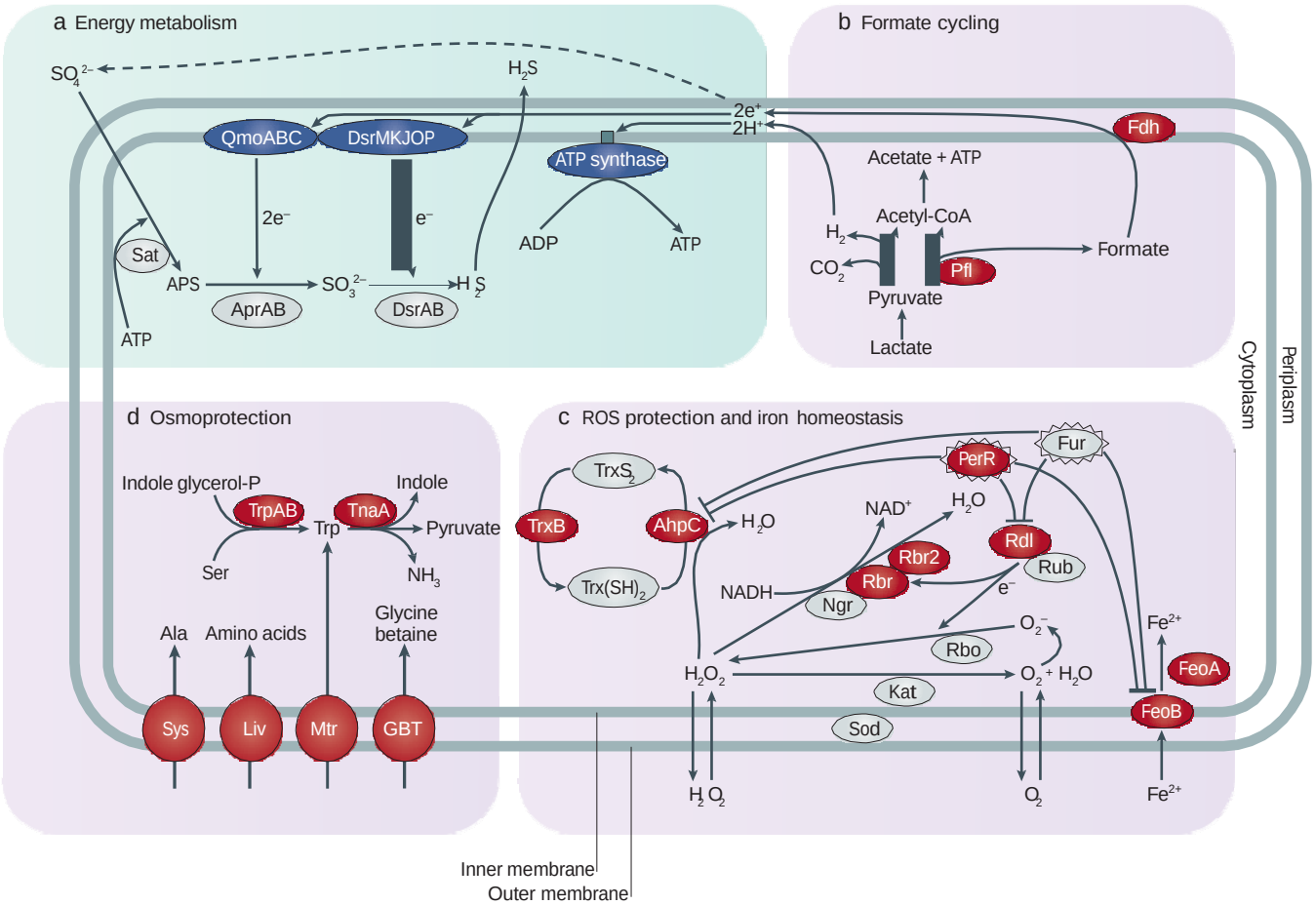


Figure 3 | **Stress response pathways in *Desulfovibrio vulgaris*. a** | Various stress responses, such as the one triggered by nitrite, result in the repression of energy metabolism by downregulation of the ATP synthase, the quinone-interacting membrane-bound oxidoreductase (QmoABC) and the transmembrane dissimilatory sulphite reductase (Dsr) transmembrane complex (DsrMKJOP), which are essential for the oxidative phosphorylation pathway that is linked to sulphate (SO_4^{2-}) reduction and hydrogen cycling. **b** | The energy and reducing equivalents that are required for stress-alleviating processes (for example, the detoxification of nitrite) can be produced by an increased flow of reducing equivalents through formate or by, potentially, 'formate cycling'. This is achieved by upregulation of formate dehydrogenase (Fdh) and pyruvate formate lyase (Pfl). **c** | *Desulfovibrio vulgaris* possesses many mechanisms for protection against reactive oxygen species (ROS), such as rubredoxin (Rub), Rub oxidoreductase (Rbo), rubrerythrin (Rbr; also known as Rr), superoxide dismutase (Sod) and catalase (Kat; also known as KatA). A global transcriptional regulator, peroxide-responsive repressor (PerR), controls the expression of several genes encoding enzymes for peroxide reduction, such as alkyl hydroperoxide reductase C (AhpC), rubrerythrins (Rbr and Rbr2) and Rub-like protein (Rdl). Several of these proteins are upregulated in response to weak oxidative conditions. In addition, there seems to be an overlap or crosstalk between the PerR regulon and the ferric uptake regulator (Fur) regulon, which controls iron homeostasis. **d** | High salinity induces an upregulation of the glycine betaine/L-proline ABC transporter (GBT), leading to accumulation of the osmoprotectant glycine betaine. Long-term exposure to high salinity also induces the upregulation of proteins that are involved in amino acid metabolism and transport, such as high-affinity branched-chain amino acid ABC transporter (Liv), tryptophan-specific transport protein (Mtr), sodium/alanine symporter (Sys), tryptophanase (TnaA) and tryptophan synthase (TrpAB). Red proteins are upregulated and blue proteins are downregulated. AprAB, APS reductase (also known as ApsAB); APS, adenosine phosphosulphate; Feo, ferrous iron transport protein; Ngr, nigerythrin; Sat, sulphate adenylyltransferase; Trx, thioredoxin; TrxB, Trx reductase.

attributed to the specific stress resistance mechanisms that are activated, which involve ATP-dependent transporters for the expulsion and import of ions[37,55].

Defence against reactive oxygen species. Because of the importance of O_2 to the survival and distribution of SRMs as anaerobes[58], biochemical pathways that confer resistance to oxidative stress and reactive oxygen species (ROS) have been the focus of various studies. In addition, *Desulfovibrio* spp. can use O_2 for growth or for detoxification[59]. These microorganisms possess a surprisingly large diversity of ROS protection mechanisms (FIG. 3c), including a unique set of proteins that consists of rubredoxin oxidoreductase (Rbo), rubredoxin–oxygen oxidoreductase (Roo) and rubrerythrin (Rbr; also known as Rr)[60,61], all of which are conserved in

SRMs[53] and provide mechanisms that scavenge ROS without regenerating intracellular O_2 — a feature that is highly desirable for anaerobic organisms. SRMs also possess ROS-scavenging enzymes that are common in aerobic microorganisms, such as superoxide dismutase (Sod) and catalase (Kat; also known as KatA)[24,62,63]. A global transcriptional regulator, peroxide-responsive repressor (PerR), seems to control the expression of a set of genes encoding enzymes for peroxide reduction, such as alkyl hydroperoxide reductase C (AhpC), the rubrerythrins (Rbr and Rbr2) and rubredoxin-like protein (Rdl), indicating that there is considerable complexity in the regulation of ROS defence pathways in *D. vulgaris* H.[46].

Recent genomic studies of *D. vulgaris* H. have focused on the expression of constituents of the ROS resistance machinery in response to various O_2 concentrations, as the species can be found in disturbed sediments and photosynthetic microbial mats, which possess low and high O_2 concentrations, respectively. Genes with known functions in ROS protection in other organisms, including *sodB* (encoding superoxide dismutase) and *kat*, were constitutively expressed, probably as a baseline protection[48,50,51]. By contrast, the expression of ROS protection genes in the PerR regulon was dynamic, being higher at weak oxidative-stress conditions (0.1% O_2 and 1mM H_2O_2) and lower in severe conditions (21% and 100% O_2)[48–52,64]. Genes that are involved in protein repair and degradation were particularly upregulated in severe oxidative-stress conditions, suggesting a shift in the response strategy from ROS elimination to the prevention of further oxidative damage[48,49]. Thus, *D. vulgaris* H. seems to tackle low levels of O_2 exposure and weak oxidative stress using mechanisms that rely on baseline protection by constitutive ROS-detoxifying enzymes (such as Sod, Kat, superoxide reductase (Sor) and Rbr), enhanced by a few additional mechanisms such as those regulated by PerR. The PerR regulation of the ROS defence system in *D. vulgaris* H. is distinct from that in *E. coli*, which uses H_2O_2-inducible genes activator (OxyR) and the superoxide-stress response regulator SoxRS[65] — two different transcriptional regulators that are unrelated to PerR — under oxidative-stress conditions. By comparison, the regulation of the oxidative-stress response does rely on PerR in *B. subtilis*, but the constituents of the PerR regulon differ significantly between *B. subtilis* and *D. vulgaris* H.[66].

Interestingly, although genes in the PerR regulon are considered to be specifically involved in resistance to oxidative stress in *D. vulgaris* H. and other bacteria[46,67], they are repeatedly upregulated under many other stress conditions tested on *D. vulgaris* H.[38–40,48,51,53] (FIG. 3c). The responses to oxidative stress also overlap, by co-regulation, with the responses to other stresses in *E. coli* and *B. subtilis*[1,3,68], but the upregulation of the PerR regulon across various stress conditions in *D. vulgaris* H. nevertheless suggests that there are additional regulatory mechanisms that remain to be identified. Given the paramount importance of O_2 and oxidative stress to the ecophysiology of *D. vulgaris* H., there could be adaptive advantages in the anticipatory expression of oxidative-stress response pathways in the event of environmental perturbations; indeed, the strategy of anticipatory expression has been shown to confer persistence on other microorganisms[69].

Osmoprotection. Fluctuations in salinity are common in many environments in which *D. vulgaris* thrives, as a result of the natural hydration–dehydration cycles that occur. The primary mechanism used by *D. vulgaris* H. for countering short-term exposure (4 hours) to high concentrations of NaCl or KCl is the transport and accumulation of osmoprotectants such as glycine betaine[37], which is one of the most widespread osmoprotectants in the environment and is found in animals, plants and microorganisms[70] (FIG. 3d). The upregulation of the glycine betaine/**l**-proline ABC transporter system (encoded by the loci DVU2297–DVU2299) and the accumulation of glycine betaine in the cytoplasm of *D. vulgaris* H.[37] resemble the saline-stress responses of other bacteria[71]. Responses to long-term exposure (100 hours) to high salinity, however, also include an upregulation of amino acid metabolism and transport genes. This suggests that the biosynthesis and transport of amino acids, which can function as osmoprotectants, provides enhanced protection against long-term exposure to high salinity in *D. vulgaris* H.[38], although the complete metabolic pathways involved remain to be elucidated.

The significance of osmoprotectants in alleviating hypersaline stress is also demonstrated in the genetic changes that enable *D. vulgaris* H. to adapt to persistent high salinity. Growth under constant salt stress (>100 mM NaCl) improved the fitness of *D. vulgaris* H. in high salinity after 100 generations, and stable salt-resistant mutants were observed after ~1,000 generations (A.Z. and J.Z., unpublished observations). Comparisons of the genome sequences of the ancestral and evolved strains revealed several mutations and deletions that were unique to the salt-adapted strains. Genome sequencing and metabolic analyses further revealed that the resistance mechanisms used in resistance to short-term salt stress, such as the influx of osmoprotectants, were genetically enhanced in the salt-evolved strains, suggesting that osmoprotectants have a key role in the alleviation of high-salinity stress.

Iron homeostasis. Genes under the control of ferric-uptake regulator (Fur) differ between *D. vulgaris* H., *B. subtilis* and other bacteria, but they generally have important roles in iron uptake and homeostasis[67,72]. As iron is an important constituent of many of the proteins involved in oxidation–reduction processes, increases in the concentration of these proteins may be correlated with higher expression of genes in the Fur regulon to enhance iron uptake. An example of this phenomenon is the simultaneous upregulation of genes in the Fur regulon and many genes encoding iron-containing proteins during the nitrite stress response in *D. vulgaris* H.[40]. However, genes in the Fur regulon are also upregulated in response to all other stress conditions that have been tested in this organism[37–40,48,51,53–55]. As it is unlikely that all of these conditions would result in iron limitation, a possible explanation is that the regulation of the PerR

Regulon
A set of genes or operons that are regulated by the same regulatory protein.

and Fur regulons may overlap owing to their similar regulatory mechanisms[46,67].

Despite the overlap of stress response pathways, as discussed above, a divergence in responses is also evident in *D. vulgaris* H.[39]. Stress-specific responses include the upregulation of the *hcp* gene (encoding hydroxylamine reductase) during nitrite stress, the Na^+/H^+ antiporter gene (*nhaC2*) in alkalinity stress, and genes involved in exopolysaccharide biosynthesis in biofilms during growth on a steel surface[73]. However, much of the divergence in stress responses is attributable to changes in the expression of a large number of genes with unknown functions, many of which genes are unique to SRMs[74]. Thus, a refined understanding of the specificity of stress responses warrants efforts to characterize these genes of unknown functions.

Syntrophic interactions and evolution of SRMs

All microorganisms live in communities, in which they can compete, cooperate or be preyed upon. However, most physiological studies of microorganisms are carried out on pure cultures, so little is known about how interspecific interactions (for example, mutualism) impose or alleviate stress on microbial populations. In particular, some SRMs engage in a remarkable type of cooperative interaction, known as syntrophy, with hydrogen-consuming archaea[75]. This interaction was first discovered between a *Desulfovibrio* sp. and a hydrogenotrophic methanogen[76]. Syntrophy literally means 'feeding together' and refers to any interaction in which two species complete a metabolic reaction from which neither species can gain energy without the cooperation of the other[75].

To explore how the physiology of *Desulfovibrio* spp. is affected by the challenge of syntrophy, a model syntrophic interaction was developed involving *D. vulgaris* H. and *Methanococcus maripaludis* S2, a hydrogenotrophic methanogen[77]. In media without an electron acceptor, *D. vulgaris* H. cooperated by transferring H_2, a waste product of lactate fermentation, to *M. maripaludis* and in return benefited from a chemical environment (a low H_2 concentration) in which lactate fermentation was thermodynamically favourable (BOX 3). The primary role of H_2, as opposed to formate, as an electron carrier was predicted by a flux balance analysis of the association[77]. This prediction was confirmed by the observation of H_2 transfer — but not formate transfer — and by comparable syntrophic growth of a *M. maripaludis* mutant lacking the ability to metabolize formate. A comparison of gene expression in *D. vulgaris* H. growing in syntrophy and in sulphate-limiting conditions, together with subsequent analyses of *D. vulgaris* H. hydrogenase mutants, suggested that this organism has a dedicated system for syntrophic growth that requires an active Coo hydrogenase and high-molecular-weight cytochrome (Hmc)[78] — another example of the central role of energy metabolism in the ecological flexibility of SRMs.

The capacity for these model syntrophic organisms to evolve improved growth was also explored. *Desulfovibrio* and *Methanococcus* spp. may have only occasionally relied on syntrophy for survival or may have evolved with multiple syntrophic partners; in particular, it is unlikely that the model strains of *D. vulgaris* and *M. maripaludis* are fully adapted to syntrophy or to living with each other, as they were originally isolated from very different environments[9,79]. Two factors may limit the growth of the microorganisms in this nascent syntrophic association, causing conditions that could result in rapid evolution[80–82]. First, the two partners suffer from low levels of energy being available for growth when they are relying on syntrophy for survival, in comparison to the growth conditions in pure culture[75]. Second, the ability of these organisms to access their energy source depends on the distribution and continued cooperation of their partner species. This situation could lead to unstable growth, especially if one species is inhibited[83–85]. A recent experiment with 24 independently evolving co-cultures confirmed these predictions[86]. Initially, growth was unstable and two co-cultures almost went extinct. By 300 generations, however, growth of the remaining co-cultures stabilized, and it was 80% faster and produced about 30% more cellular material than the growth of the ancestors. Analysis of the growth of mixed-ancestry co-cultures indicated that both species acquired mutations contributing to the improved productivity. These results demonstrate that improved stability and productivity are typical adaptations to the initial stress of living in a community, and that adaptive changes can be rapid.

System-level analyses of *D. vulgaris* H. in model communities are currently being extended to incorporate additional species[87] and identify mutations that confer improved syntrophic growth. Recently developed strategies for identifying the source species of specific proteins in flux analyses of mixed populations should facilitate research on how the metabolism — and stress response — of one microbial partner is affected by the presence of other microorganisms. This approach will provide a deeper understanding of the physiology of microbial growth in community contexts[88].

Metagenomics of SRMs in natural environments

SRMs have extraordinarily large numbers of genes and pathways involved in the response to environmental stresses, and this probably contributes to the adaptation of these organisms to diverse habitats. The first step towards understanding this adaptation is to undertake studies to detect, characterize and quantify SRMs in natural microbial communities. Such efforts have traditionally been hampered by the diversity and as-yet-uncultivated status of many SRMs, but these studies have recently been transformed by the development of large-scale genome sequencing and associated metagenomic technologies, such as functional gene arrays. The new techniques have been used to characterize SRMs in various environments, such as fresh water[89], deep-sea sediments and vents[90,91], a gold mine[31], symbionts[92,93], animal microbiomes[94–96] and groundwater[97]. Owing to space limitations, this section focuses primarily on representative extreme environments and in particular on heavy-metal-contaminated groundwater.

Flux balance analysis
Mathematical modelling of the flux of metabolites through metabolic networks, which can be as complex as the total metabolic capacity encoded by a genome.

Functional gene arrays
Microarrays that contain probes targeting sequences which are unique to genes within families of interest. For example, these may be genes encoding enzymes that are involved in antibiotic resistance, energy metabolism, stress responses, the degradation of organic contaminants or the biogeochemical cycles of carbon, nitrogen, phosphorus, sulphur and various metals, or they may be genes from phages or human pathogens.

Box 3 | **Mutually beneficial interactions involving metabolite exchange**

Archaeal and bacterial species can engage in a variety of mutually beneficial interactions in which a metabolite of one population can be a nutrient for another. Cross-feeding of metabolites within a population evolves readily[152] and probably occurs in every community. For example, in eutrophic lakes, non-motile photosynthetic bacteria provide excess fixed organic carbon to attached betaproteobacteria in exchange for motility to the optimal light and chemical environment within the lake[153]. In anaerobic environments lacking appropriate electron acceptors, such as lake sediments and anaerobic digestors, a specialized mutualism called syntrophy is responsible for the final stages of carbon oxidation[75]. In these associations, an end product that inhibits energy generation from fermentation in one species is consumed by a second species, allowing both species to gain energy. These syntrophic interactions often involve methanogenic archaea that consume intermediates produced by firmicutes such as *Desulfotomaculum*, or by deltaproteobacteria such as *Desulfovibrio* or *Syntrophobacter* spp.[75]. Syntrophic associations may degrade a variety of compounds, including ethanol, fatty acids, propionate, butyrate, benzoate and organic acids such as lactate. In one consortium that was developed in the laboratory, *Geobacter sulfurreducens* and *Wolinella succinogenes* cooperated to degrade acetate using nitrate as an electron acceptor[154]. The transferred end product in syntrophies can be H_2, formate or cysteine[154]. These metabolites can be transferred between two or more species by diffusion through the environment (as in the model syntrophy established between *Desulfovibrio vulgaris* and *Methanococcus maripaludis*) or within dense aggregates of cells. In the case of diffusion within cell aggregates, particular strains or species can become specialized to one another such that they cannot be grown separately. For example, two species from the genus *Geobacter* that initially exchanged H_2 or formate during syntrophic metabolism of ethanol were found to evolve (after being co-cultured for 660 generations) the direct transfer of electrons through cytochrome-coated pili as a more efficient way of relieving end product inhibition[155]; moreover, the two microorganisms became obligate syntrophs. In other syntrophies, cellular appendages can mediate communication between the two partners: the tip of the flagella of *Pelotomaculum thermopropionicum* induces substantial changes in gene expression in its partner, *Methanothermobacter thermautotrophicus*[153].

Detection of SRMs using microarray technologies. A variety of molecular tools have been applied to the detection of SRMs in natural environments using highly conserved genes such as those coding for the small subunit (SSU) ribosomal RNA, as well as the genes *aprBA* (also known as *apsBA*) and *dsrAB*[98–100], which encode enzymes involved in sulphate reduction pathways. In comparison to SSU rRNA genes, *aprBA* and *dsrAB* can provide a higher taxonomic resolution for the detection of SRM populations in complex microbial communities.

PCR amplification-based approaches have been used to study the abundance, diversity and composition of SRMs from different habitats[101–106]. However, high-throughput sequencing and associated metagenomic technologies, such as phylogenetic oligonucleotide arrays and functional gene arrays[107], are more powerful for providing a comprehensive view of SRM diversity and sulphate reduction processes in natural environments[15,97,108–111]. One of these technologies, GeoChip, is a functional gene array that contains probes targeting key genes involved in microbial functional processes such as virulence, stress responses, biogeochemical cycling of carbon, nitrogen, sulphur, phosphorus and metals, and biodegradation of environmental contaminants. GeoChip allows the analysis of the functional diversity, composition, structure and activities of microbial communities, as well as the investigation of the links between community structure and ecosystem functioning[15,109,112]. The latest version of GeoChip (GeoChip 4.0) contains probes for >3,000 *dsrAB* and >500 *aprAB* genes, and targets >41,000 genes from 45 gene families that are involved in various types of environmental stress[112].

The use of GeoChip to study microbial communities in uranium-contaminated groundwater has shown that SRMs play a major part in reduction of uranium vi[15]. The abundances of these indigenous SRMs are increased with the injection of ethanol as a carbon substrate and decreased by increased dissolved O_2 (REFS 113,114). GeoChip has also been applied to the study of hydrothermal vents, for which it indicated the presence of very diverse SRM populations that, along with other microorganisms, undergo rapid dynamic succession and adaptation to the steep temperature and chemical gradients across the vent chimney [115]. In addition, SRMs were detected in deep-sea basalts, suggesting the occurrence of anaerobic processes in these extremely nutrient-poor environments[116]. More recently, GeoChip has been used to investigate microbial responses to the oil spill in the Gulf of Mexico[112]. SRM populations were found to be considerably larger in the oil-contaminated samples than in non-contaminated samples (Z. Lu and J.Z., unpublished observations), suggesting that these organisms contribute to natural bioremediation of oil-contaminated deep-sea ecosystems, as indicated by previous studies[117–120]. These and other applications of GeoChip[109,121–124] demonstrate that this is a powerful tool for detecting and monitoring SRM populations and their associated microbial communities, as well as for assessing their metabolic potential and activity in response to different environmental stresses.

Complementary to GeoChip, phylogenetic oligonucleotide arrays based on 16S rRNA genes (for example, PhyloChip) provide phylogenetic information about SRMs in the environment[108,110,111]. One of these microarrays, SRP-PhyloChip, was first developed to detect SRMs in periodontal tooth pockets and in the chemocline of a hypersaline cyanobacterial mat from Solar Lake, Sinai, Egypt[111]. Another study with SRP-PhyloChip showed that floodplain soils harboured distinct SRM communities with characteristic biogeographical patterns and that the distribution of several SRMs (including species from the genera *Desulfosarcina*, *Desulfomonile* and *Desulfobacter*) varied according to salinity and the presence of plant nutrients[125]. PhyloChip has also been used to detect other microorganisms in a variety of environments, such as contaminated sites[108,126].

Metagenomics of SRMs in heavy-metal-contaminated sites. Another area of intense study concerns the potential use of SRMs for the bioremediation of legacy wastes by the reductive immobilization of radionuclides and heavy metals. One site with such a legacy waste is the US Department of Energy Field Research Center (FRC), located in Oak Ridge, Tennessee. The local groundwater in the vicinity of the site contains one of the most concentrated mobile, subsurface uranium plumes in

Chemocline
The interface region with a sharp vertical chemical gradient in a body of water. In this case, it refers to an O_2 gradient, which is caused by the production of O_2 by the cyanobacteria in a mat.

the United States. Numerous SRM species (particularly from the Deltaproteobacteria and the Firmicutes) have been detected at various locations within the FRC site[97,108,113,127–141], suggesting that SRMs have successfully adapted to this environment.

A recent metagenomic analysis has compared the distribution of species in contaminated and pristine groundwater areas within the FRC site[97]. An indigenous microbial community composed of 4–10 species, dominated by denitrifying betaproteobacteria and deltaproteobacteria, was detected at the contaminated area studied, which is one of the most highly contaminated areas at the FRC (with a pH of ~3.7 and high concentrations of uranium, nitrate, sulphate, chlorinated organic compounds and aromatics). Despite the high concentration of sulphate in the environment, SRMs constituted only a minor fraction of the total biomass, and no complete gene sets for dissimilatory sulphate reduction pathways were identified[97]. Analysis of the metagenome from the pristine area indicated the presence of sulphate-reducing deltaproteobacteria at low abundance, with the orders Desulfuromonadales and Myxococcales as the dominant deltaproteobacterial lineages. The elimination of nitrate stress by denitrification seemed to stimulate the growth of SRMs at the contaminated site[113,131,133,137,139], in agreement with functional genomic studies that have shown that nitrate is a potent inhibitor of these organisms[39]. These results underscore the value of stress response analysis for improving the effective implementation of SRMs in biotechnological applications.

Concluding remarks and future perspectives

The application of high-throughput genomic tools using *D. vulgaris* H. as a model has provided crucial system-level insights into the strategies that are used by SRMs to cope with adverse environmental conditions. First, shifting energy metabolism appears to be an important strategy in stress responses and the establishment of syntrophy. The sensitivity of hydrogen cycling to stress supports the view that hydrogen cycling has a central role in the energy metabolism of *D. vulgaris* H. However, it remains to be seen whether this is a common feature among SRMs, particularly given the vast diversity of genes that encode proteins involved in the energy metabolism of SRMs. Second, oxidative-stress responses have a surprisingly prevalent role in coping with both oxidative and non-oxidative stresses. This probably confers an adaptive advantage through the anticipatory expression of defence pathways against ROS, as these molecules cause the most critical stress to an anaerobe such as *D. vulgaris* H. Third, *D. vulgaris* H. activates distinct response pathways that are specific to a broad range of stresses, in agreement with comparative genomic analyses that reveal an unusually large number and diversity of response regulators involved in signal transduction. Thus, the characterization of distinct signal transduction pathways is required to understand how the microorganism senses and responds to environmental stimuli. Fourth, under laboratory conditions, *D. vulgaris* H. can grow with methanogens in a syntrophic association that can evolve enhanced stability and productivity. Although this syntrophic association may not be natural, it provides a model to investigate potential mechanisms that allow the distribution and evolution of SRMs in environments that are depleted of sulphate as the terminal electron acceptor. The remarkably broad distribution of SRMs and the adaptation of these species to various environmental niches have been confirmed by metagenomic technologies (such as PhyloChip and GeoChip). More importantly, these metagenomic analyses also reveal environmental factors that limit the activity of SRMs, such as the growth inhibition by high concentrations of nitrate, consistent with functional genomic studies of stress responses. These metagenomic analyses highlight the importance of relieving key stresses when exploiting SRMs for biotechnological applications such as heavy-metal bioremediation.

However, so far we have only scratched the surface of the biology of SRMs. More systematic, coordinated and integrated efforts are greatly needed using the next generation of 'omics' technologies. For instance, metagenomics combined with single-cell genomics will be a powerful tool for elucidating the genetic diversity of as-yet-uncultivated SRMs in a variety of environments. This strategy has proved successful in sequencing single cells of the uncultivated microorganisms present in environmental samples, even if the species of interest is not abundant[142,143]. Furthermore, one of the greatest challenges in biology is to understand how the genotype and environment interact to determine the phenotype and fitness of an organism; experimental evolution of SRMs under controlled conditions will be extremely helpful for linking subcellular molecular and metabolic processes with the evolutionary processes and functions that are observed at the population level. In addition, it is essential to determine whether an understanding of microbial community structure at the molecular level improves our predictive power concerning the ecological and evolutionary responses of microbial communities to environmental changes[144,145]. To address these questions, we need to develop robust laboratory systems with various levels of complexity to mimic the interactions among different microbial populations in natural environments (for example, syntrophic and competitive interactions). Finally, because the dynamic behaviours of biological systems at various levels (cell, individual, population, community and ecosystem) are measured on different temporal and spatial scales, the prediction of ecosystem functioning, stability and succession by linking cell-level genomic information to ecosystem-level functional information is extremely challenging. Thus, novel mathematical frameworks and computational tools are needed to achieve a system-level understanding and prediction of microbial community dynamics, behaviour and functional stability. We believe that the study of stress responses in SRMs will significantly contribute to a better understanding of the links between microbial community structure and functioning.

Single-cell genomics
The characterization of the genome of an isolated single cell (or a group of these cells) by large-scale sequencing and other high-throughput technologies. Single cells are typically isolated by optical tweezers (which use highly focused laser beams to physically manipulate microscopic objects), flow sorting or serial dilution, and these cells are then subjected to genome amplification, sequencing and/or functional measurements.

Experimental evolution
An approach to studying evolution that involves the propagation of populations for many generations in controlled and reproducible environmental conditions, and the observation of the phenotypic and genetic changes in those populations.

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