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

Author:
Zhou, J.

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


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.

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, Bacillus subtilis and Saccharomyces cerevisiae, 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, 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. SRMs are characterized by the ability to carry out dissimilatory sulphate reduction (that is, energy generation by coupling the oxidation of organic compounds or H₂ to the reduction of sulphate (SO₄²⁻) to sulphide (S⁻) and other sulphur-containing compounds), which directly links these organisms to the natural cycling of both carbon and sulphur. 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. 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.

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

Figure 1 | Sulphate-reducing microorganisms and the carbon and sulphur cycles. Sulphate-reducing microorganisms (SRMs) use sulphate (SO\textsubscript{4}\textsuperscript{2-}) as the terminal electron acceptor during the degradation of simple organic matter. This reduction of SO\textsubscript{4}\textsuperscript{2-} produces hydrogen sulphide (H\textsubscript{2}S) and carbon dioxide (CO\textsubscript{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\textsubscript{4}\textsuperscript{2-} reduction, H\textsubscript{2}S can be subsequently oxidized by chemolithotrophic organisms to elemental sulphur (S\textsubscript{0}) and further to SO\textsubscript{3}\textsuperscript{2-}. Sulphate can also be derived from atmospheric deposition of sulphur oxides that are formed from the chemical oxidation of H\textsubscript{2}S. Subsequently, SO\textsubscript{3}\textsuperscript{2-} can be again reduced by SRMs to H\textsubscript{2}S, 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\textsubscript{2}S. Other biotransformations in the sulphur cycle include the reduction of S\textsubscript{0} to H\textsubscript{2}S and sulphur disproportionation, in which S\textsubscript{0} is converted into both H\textsubscript{2}S and SO\textsubscript{3}\textsuperscript{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\textsubscript{4}\textsuperscript{2-} reduction. CO\textsubscript{2}, one of the end products of SO\textsubscript{4}\textsuperscript{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\textsubscript{4}\textsuperscript{2-} reduction or other modes of metabolism.

Radionuclides such as chromium and uranium\textsuperscript{3,21,22} 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\textsuperscript{22,23}. 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 \textit{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\textsuperscript{24-31}. Sequences are not yet available for SRMs that represent other major lineages, such as the crenarchaeotal genera \textit{Caldivirga} and \textit{Thermococcus}, or for the recently isolated \textit{Thermodesulfobium naragense}, 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\textsuperscript{22}. 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 ecosystems144,146. 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)37,39,51,149. At the community scale, high-throughput metagenomic technologies, such as large-scale genome sequencing97, GeoChip15,109 and PhyloChip16, 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 pathways36), by the development of models for cellular stress responses37,48,51,147,148 and for the evolutionary trajectories of genes86, pathways, cells, populations and communities97, and for cellular and community networks91,152, and possibly by mathematical modelling, simulation and prediction of stress responses across different organizational levels such as cells, populations and communities).

![Image](48x277 to 155x350)

![Image](48x405 to 155x437)

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.

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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 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, rRNA 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. 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>
<thead>
<tr>
<th>Organism</th>
<th>Temperature*</th>
<th>Genomic size (Mb)</th>
<th>% GC content</th>
<th>Habitat</th>
<th>Characteristics</th>
<th>Accession‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Domain Archaea, phylum Euryarchaeota, class Archaeoglobi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Archaeoglobus fulgidus DSM 4304</td>
<td>T (83 °C)</td>
<td>2.18</td>
<td>48.58</td>
<td>Geothermal vents</td>
<td>Archaeal model of sulphate-reducing microorganisms</td>
<td>NC_000917</td>
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<tr>
<td>Archaeoglobus profundus DSM 5631</td>
<td>T (82 °C)</td>
<td>1.56</td>
<td>42.00</td>
<td>Geothermal vents</td>
<td>Mixotrophic strain requiring H₂ and acetate for growth</td>
<td>NC_013741</td>
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<tr>
<td><strong>Domain Bacteria, phylum Firmicutes, class Clostridia</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Candidatus Desulfuris audaxviator MP104C'</td>
<td>T (60 °C)</td>
<td>2.35</td>
<td>60.85</td>
<td>Deep subsurface (2.8 km depth) in a gold mine</td>
<td>Forms single-species communities in the deep subsurface</td>
<td>NC_010424</td>
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<tr>
<td>Desulfotomaculum acetoxidans DSM 771</td>
<td>M (36 °C)</td>
<td>4.55</td>
<td>41.55</td>
<td>Fresh water, ocean or animal waste</td>
<td>Oxidizes acetate to CO₂</td>
<td>NC_013216</td>
</tr>
<tr>
<td>Desulfotomaculum reducens MI-1</td>
<td>M (37 °C)</td>
<td>3.61</td>
<td>42.28</td>
<td>Heavy-metal-contaminated sediment</td>
<td>Gram-positive model of sulphate-reducing microorganisms; reduces chromium and uranium</td>
<td>NC_009253</td>
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<tr>
<td><strong>Domain Bacteria, phylum Nitrospirae, class Nitrospira</strong></td>
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<tr>
<td>Thermodesulfovibrio yellowstonii DSM 11347</td>
<td>T (65 °C)</td>
<td>2.00</td>
<td>34.13</td>
<td>Hot springs</td>
<td>Thermophile</td>
<td>NC_011296</td>
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<tr>
<td><strong>Domain Bacteria, phylum Proteobacteria, class Deltaproteobacteria</strong></td>
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<tr>
<td>Desulfatibacillum alkenivorans AK-01</td>
<td>M (30 °C)</td>
<td>6.52</td>
<td>54.48</td>
<td>Oil-polluted sediment</td>
<td>Degrades alkenes</td>
<td>NC_011768</td>
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<tr>
<td>Desulfobacterium autotrophicum HRM2</td>
<td>M (30 °C)</td>
<td>5.66</td>
<td>48.76</td>
<td>Ocean</td>
<td>Marine autotroph</td>
<td>NC_012108</td>
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<tr>
<td>Desulfococcus oleovorans Hxd3</td>
<td>M (30 °C)</td>
<td>3.94</td>
<td>56.17</td>
<td>Oil–water mixtures from oil production plants</td>
<td>Degrades alkanes anaerobically</td>
<td>NC_009943</td>
</tr>
<tr>
<td>Desulfotalea psychrophila LSv54</td>
<td>P (10 °C)</td>
<td>3.66</td>
<td>46.63</td>
<td>Ocean</td>
<td>Marine psychrophile</td>
<td>NC_006138</td>
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<tr>
<td>Desulfobacterium retbaense DSM 5692</td>
<td>M (37 °C)</td>
<td>2.91</td>
<td>57.33</td>
<td>Hypersaline lake sediment</td>
<td>Halophile</td>
<td>NC_013223</td>
</tr>
<tr>
<td>Desulfonatronospira thiodismutans ASO3-1§</td>
<td>M (36 °C)</td>
<td>3.97</td>
<td>51.33</td>
<td>Hypersaline lake sediment</td>
<td>Halophile</td>
<td>ACJN00000000</td>
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<tr>
<td>Desulfoclasticum baculatum DSM 4028</td>
<td>M (36 °C)</td>
<td>3.94</td>
<td>58.65</td>
<td>Manganese ore</td>
<td>Metabolizes H₂ very efficiently</td>
<td>NC_013173</td>
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<tr>
<td>Desulfovibrio aspoeensis Aspo-2§</td>
<td>M (30 °C)</td>
<td>3.57</td>
<td>62.70</td>
<td>Deep groundwater</td>
<td>Lives in a nutrient-poor environment</td>
<td>ADDI00000000</td>
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<tr>
<td>Desulfovibrio desulfuricans subsp. desulfuricans G20</td>
<td>M (36 °C)</td>
<td>3.73</td>
<td>57.84</td>
<td>Soil</td>
<td>Has strong bioremediation potential</td>
<td>NC_007519</td>
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<tr>
<td>D. desulfuricans subsp. desulfuricans ATCC 27774</td>
<td>M (37 °C)</td>
<td>2.87</td>
<td>58.07</td>
<td>Soil</td>
<td>Reduces nitrate</td>
<td>NC_011883</td>
</tr>
<tr>
<td>Desulfovibrio magneticans RS-1</td>
<td>M (36 °C)</td>
<td>5.32</td>
<td>62.67</td>
<td>Soil</td>
<td>Forms magnetosomes</td>
<td>NC_012795</td>
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<tr>
<td>Desulfovibrio piger ATCC 29098§</td>
<td>M (36 °C)</td>
<td>2.83</td>
<td>63.05</td>
<td>Human digestive tract</td>
<td>Commensal of humans</td>
<td>ABXU00000000</td>
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<td>Desulfovibrio salexigens DSM 2638</td>
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<td>4.29</td>
<td>47.09</td>
<td>Marine sediment</td>
<td>Halophile</td>
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<td>Desulfovibrio sp. FW1012B§</td>
<td>M (37 °C)</td>
<td>4.18</td>
<td>66.00</td>
<td>Uranium-contaminated groundwater</td>
<td>Isolated from biostimulated, uranium-contaminated groundwater</td>
<td>ADFE00000000</td>
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<tr>
<td>Desulfovibrio fructosovorans J§</td>
<td>M (37 °C)</td>
<td>4.67</td>
<td>63.00</td>
<td>Estuarine sediment</td>
<td>Can metabolize fructose</td>
<td>AECZ00000000</td>
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</table>
systems in which the signal nitrogen limitation. A protein in bacteria that enables binding of RNA polymerase to gene promoters enables binding of RNA polymerase to gene promoters. A second messenger that is used in signal transduction in a wide variety of bacteria. Transcription factor σ54 A protein in bacteria that enables binding of RNA polymerase to gene promoters specifically in response to nitrogen limitation.

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

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<th>Characteristics</th>
<th>Accession†</th>
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<td>3.77</td>
<td>63.28</td>
<td>Soil</td>
<td>Gram-negative model of sulphate-reducing microorganisms</td>
<td>NC_002937</td>
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<tr>
<td>Hildenborough</td>
<td></td>
<td></td>
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<tr>
<td>D. vulgaris DP4</td>
<td>M (36 °C)</td>
<td>3.66</td>
<td>63.16</td>
<td>Freshwater lake sediment</td>
<td>Lacks insertion elements that are present in D. vulgaris Hildenborough</td>
<td>NC_008741</td>
</tr>
<tr>
<td>D. vulgaris RCH1§</td>
<td>M (36 °C)</td>
<td>3.70</td>
<td>63.00</td>
<td>Chromium-contaminated groundwater</td>
<td>Sequenced for comparative analysis</td>
<td>NA</td>
</tr>
<tr>
<td>D. vulgaris ‘Miyazaki F’</td>
<td>M (36 °C)</td>
<td>4.04</td>
<td>67.00</td>
<td>Degraded paddy field</td>
<td>Well-characterized hydrogenase</td>
<td>NC_011769</td>
</tr>
<tr>
<td>Syntrophobacter fumaroxidans</td>
<td>M (36 °C)</td>
<td>4.99</td>
<td>60.00</td>
<td>Anaerobic sludge</td>
<td>Syntrophic; degrades propionate</td>
<td>NC_008554</td>
</tr>
<tr>
<td>MPOB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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).

**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 σ54

A protein in bacteria that enables binding of RNA polymerase to gene promoters specifically in response to nitrogen limitation.

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 σ54 (also known as RpoN). Transcription factor σ54 is essential in two deltaproteobacteria: Myxococcus xanthus and Geobacter sulfurreducens. The unusually large number of σ54-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...
the growth of this microorganism despite the energetic constraints that are associated with sulphate reduction (see the figure). According to this model, hydrogen cycling is not necessary for the generation of additional ATP for growth. CO is metabolized in the periplasm, where it 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 network. From here, electrons are proposed to be transferred to the menaquinone-linked quinone reductase complex (Qrc) 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 (FIG. 3a), which removes the pyrophosphate (PP) 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. The process is made energetically favourable by the activity of inorganic pyrophosphatase, which removes the pyrophosphate (PP) 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. CO is metabolized in the cytoplasm by CO dehydrogenase, and formate is cycled to the periplasm, where it is metabolized by formate dehydrogenase (Fdh). Hydrogen cycling is not necessary when H2 is used as the electron donor as periplasmic metabolism of H2 directly establishes the electrochemical gradient that is necessary for ATP synthesis.

### 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 O2, H2O2, NaCl, KCN, nitrite salts, nitrate salts, heat shock, starvation, and alkaline pH.

### 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. Indeed, genes that are involved in energy metabolism, such as those encoding the ATP synthase, membrane hydrogenases and the DsrMKJOP transmembrane complex, 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. This problem is resolved in *D. vulgaris* H. by increasing the flow of reducing equivalents through formate or by, potentially, carrying out a 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 H2 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 of the environmental *Desulfovibrio* spp. isolates. 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.
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 (SO₂⁻) 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 ions³⁷,³⁸.

Defence against reactive oxygen species. Because of the importance of O₂ to the survival and distribution of SRMs as anaerobes³⁸, 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 O₂ for growth or for detoxification⁶⁰. 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\(^4\) 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)\(^{24,62}\). 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 ruberythrin (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\(^{48,30,51}\). 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\(^{49,49}\). 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\(^5\) — 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.\(^46\).

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.\(^30–40,48,51,53\) (FIG. 3c). The responses to oxidative stress also overlap, by co-regulation, with the responses to other stresses in \(E. coli\) and \(B. subtilis\)\(^34,54\), 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\(^6\).

Osmoprotection. Fluctuations in salinity are common in many environments in which \(D. vulgaris\) H. 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\(^39\) (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\(^4\).

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.\(^37\), 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\(^{62,72}\). 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.\(^48\). 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
and Fur regulons may overlap owing to their similar regulatory mechanisms.

Despite the overlap of stress response pathways, as discussed above, a divergence in responses is also evident in D. vulgaris H. 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. 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. 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. This interaction was first discovered between a Desulfovibrio sp. and a hydrogenotrophic methanogen. 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.

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. In media without an electron acceptor, D. vulgaris H. cooperated by transferring H2, a waste product of lactate fermentation, to M. maripaludis and in return benefited from a chemical environment (a low H2 concentration) in which lactate fermentation was thermodynamically favourable (Box 3). The primary role of H2, as opposed to formate, as an electron carrier was predicted by a flux balance analysis of the association. This prediction was confirmed by the observation of H2 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) — 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. Two factors may limit the growth of the microorganisms in this nascent syntrophic association, causing conditions that could result in rapid evolution. 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. 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. A recent experiment with 24 independently evolving co-cultures confirmed these predictions. 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 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.

**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, deep-sea sediments and vents, a gold mine, symbionts, animal microbiomes, and groundwater. Owing to space limitations, this section focuses primarily on representative extreme environments and in particular on heavy-metal-contaminated groundwater.
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 aprB and dsrA. These metabolic pathways can be transferred between two species or more 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 H₂ or formate 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.

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 microbial communities, as well as the investigation of the links between community structure and ecosystem functioning. 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 genera that are involved in various types of environmental stress.

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. The abundances of these indigenous SRMs are increased with the injection of ethanol as a carbon substrate and decreased by increased dissolved O₂. 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. In addition, SRMs were detected in deep-sea basals, suggesting the occurrence of anaerobic processes in these extremely nutrient-poor environments. More recently, GeoChip has been used to investigate microbial responses to the oil spill in the Gulf of Mexico, and GeoChip populations were found to be considerably larger in the oil-contaminated samples than in non-contaminated samples. GeoChip and other applications of GeoChip 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. One of these microarrays, SRP-PhyloChip, was first developed to detect SRMs in periodontal tooth pockets and in the chemoclaine of a hypersaline cyanobacterial mat from Solar Lake, Sinai, Egypt. 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, Desulfomona and Desulfobacter) varied according to salinity and the presence of plant nutrients. PhyloChip has also been used to detect other microorganisms in a variety of environments, such as contaminated sites.

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 and probably occurs in every community. For example, in eutrophic lakes, non-motile, photosynthetic bacteria can provide excess fixed organic carbon to attached betaproteobacteria in exchange for motility to the optimal light and chemical environment within the lake. 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. These syntrophic interactions often involve methanogenic archaea that consume intermediates produced by firmicutes such as Desulfotomaculum, by deltaproteobacteria such as Desulfovibrio or Syntrophobacter spp. 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. The transferred end product in syntrophies can be H₂, formate or cysteine. 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 H₂ 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; 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.

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the United States. Numerous SRM species (particularly from the Deltaproteobacteria and the Firmicutes) have been detected at various locations within the FRC site\textsuperscript{105,106,113,127-141}, 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\textsuperscript{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 \(\sim 3.7\) 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\textsuperscript{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\textsuperscript{115,117,119,125} in agreement with functional genomic studies that have shown that nitrate is a potent inhibitor of these organisms\textsuperscript{98}. 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\textsuperscript{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\textsuperscript{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 function.


This study shows that most of the recently acquired histidine kinase domains in B. vulgaris have arisen by lineage-specific expansion, and that these genes are more likely to be present as orphan proteins, separate from their cognate partner.


104. Hillesland, K. L. & Stahl, D. A. Rapid evolution of microbial communities. Proc. Natl Acad. Sci. USA 107, 2123–2129 (2010). The first example of sympathy evolution observed in real time. This paper is a good example of how rapid evolution can be observed in microbial consortia.
107. This study develops a strategy that allows the use of isotopomer-based flux analysis to study bacterial mixed cultures, such as the D. vulgaris—M. maripaludis co-culture.
111. Hazen, T. et al. Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 310, 204–208 (2001). This paper details the use of GeoChip 4.0 and other technologies to examine the potential of indigenous microbial communities to degrade contaminants from the oil spill in the Gulf of Mexico.


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