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MINIREVIEW

The Genus *Desulfovibrio*: The Centennial†

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Exactly 100 years ago W. M. Beyerinck reported the isolation of *Spirillum desulfuricans* from a Dutch city canal in Delft (6). In introducing his work, Beyerinck pointed out that the microbial formation of hydrogen sulfide is of great importance and interest from both purely scientific and applied points of view. Sewage contamination caused evolution of large amounts of hydrogen sulfide from city canals in summertime. The annual phenomenon constituted "eine wahren Schrecken" (a true horror). Beyerinck found that the levels of the "sulfidferment" were subject to seasonal changes, reaching maxima in July, August, and September. He managed to enrich the "sulfidferment" and obtained isolated colonies in agar, which were distinctive because of their surrounding, black iron sulfide precipitate. The motile, curved rod morphology that he reported for *Spirillum desulfuricans* leaves little doubt that he isolated and characterized the first *Desulfovibrio* species. In concluding his paper, Beyerinck indicated that it would be most interesting to know whether *Spirillum desulfuricans* uses electron acceptors other than sulfate and whether the species found in city canals is the same as that occurring in marine and soil environments. As a final point of interest, he suggested the study of the distribution of these bacteria in soils and sediments as a function of depth. Although progress has been made in all of these areas, these century-old questions are still valid today.

Many researchers have added to our current knowledge of gram-negative, sulfate-reducing eubacteria, the class to which *Desulfovibrio* species belong. John Postgate contributed, in addition to perceptive and diverse science, which included the discovery of cytochrome c_3, a comprehensive and popular monograph (48). Harry D. Peck Jr., Jean LeGall, and many others have documented the wide variety of redox enzymes and redox proteins that are found in *Desulfovibrio* species and have elucidated or suggested roles for these in *Desulfovibrio* metabolism (36, 46). Fritz Widdel has added depth and perspective to our understanding of gram-negative sulfate-reducing eubacteria by uncovering many other genera. These genera now include *Desulfobulbus*, *Desulfobacter*, *Desulfobacterium*, *Desulfococcus*, *Desulfomonile*, *Desulfonema*, *Desulfobotulus*, and *Desulfoarculus* (72, 73).

Following brief considerations of regular and exceptional metabolism, I will focus on the molecular biology of sulfate-reducing eubacteria in this minireview. This automatically limits me to the genus *Desulfovibrio*, because molecular biology of the other genera has not yet progressed beyond sequencing of 16S rRNA genes for classification purposes. Molecular biology has helped to definitively assign redox proteins to either the periplasm or the cytoplasm and has uncovered the sequences and thereby potential structures of membrane-bound redox protein complexes, which could provide the essential links between periplasmic and cytoplasmic redox reactions. Models based on these sequences are still tentative, but their consideration is important to stimulate thought on how free energy released in dissimilatory sulfate reduction is stored as a proton gradient or otherwise. Molecular biological studies are also starting to uncover how *Desulfovibrio* spp. find their niche chemotactically in the microbial world and are a great help in defining the enormous diversity of sulfate-reducing bacteria that are present in the environment.

DESULFOVIBRIO METABOLISM: ELECTRON DONORS AND ACCEPTORS

Sulfate is the defining electron acceptor of *Desulfovibrio* spp. It is reduced to sulfide by a series of reactions with adenosine-5′-phosphosulfate (APS) and sulfite as intermediates. Four enzymes (ATP sulfurylase, APS reductase, sulfite reductase, and pyrophosphatase [46, 48, 73]), all located in the cytoplasm, catalyze the following reaction:

$$SO_4^{2-} + ATP + 8H^+_{in} + 8e \rightarrow HS^- + AMP + 2P_i \quad (1)$$

From overall reaction 1 it is evident that reduction of sulfate requires investment of two high-energy phosphate bonds. This investment is recouped by proton-driven and/or substrate-level phosphorylation of ADP to ATP.

Hydrogen, formate, lactate or pyruvate, and many other organic compounds, including crude oil components and metallic iron, can serve as electron donors for sulfate reduction (26, 27, 46, 48, 54, 73). Chemolithotrophic growth of *Desulfovibrio* spp. with H_2 as the electron donor requires acetate and CO_2 as the carbon source and was first described and explained by Thauer and coworkers (4, 5). Hydrogen oxidation is catalyzed by periplasmic hydrogenases:

$$4H_2 \rightarrow 8H^+_{out} + 8e \quad (2)$$

From reactions 1 and 2 we obtain:

$$4H_2 + ATP + SO_4^{2-} + 8H^+_{in} \rightarrow HS^- + \quad (3)$$

$$AMP + 2P_i + 8H^+_{out}$$

Thus, a proton gradient results from reduction of sulfate by hydrogen because of the compartmentalization of the participating enzymes. If electron transport in overall reaction 3 is coupled to active export of an additional n protons:

$$nH^+_{in} \rightarrow nH^+_{out} \quad (4)$$

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† This paper is dedicated to Harry D. Peck Jr., who devoted most of his scientific career to achieving a broad understanding of the genus *Desulfovibrio*.

then overall reaction 3 changes to:

$$4H_2 + ATP + SO_4^{2-} + (8 + n)H^+_{in} \rightarrow HS^- + AMP + 2P_i + (8 + n)H^+_{out} \quad (5)$$

Proton translocation studies with washed cells of *Desulfovibrio vulgaris* to which sulfite was added under a hydrogen atmosphere have provided evidence for active transport of an additional 6 to 8 protons per mol of sulfate reduced in the sulfite reduction step (34). Determination of the growth yield of *D. vulgaris*, growing on hydrogen and sulfate as the sole energy source, suggested a net synthesis of 1 mol of ATP per mol of sulfate reduced (5). Thus, import of some or all of the $(8 + n)$ protons is coupled to the synthesis of 3 mol of ATP:

$$(8 + n)H^+_{out} + 3ADP + 3P_i \rightarrow (8 + n)H^+_{in} + 3ATP \quad (6)$$

Also, AMP and ATP can be converted to ADP:

$$AMP + ATP \rightarrow 2ADP \quad (7)$$

Combination of reactions 5 to 7 gives:

$$4H_2 + SO_4^{2-} + ADP + P_i \rightarrow HS^- + ATP \quad (8)$$

When lactate or pyruvate serves as an electron donor for sulfate reduction, it is incompletely oxidized to acetate and CO_2. Lactate is oxidized to pyruvate by an NAD(P)-independent, membrane-bound lactate dehydrogenase that has not yet been well characterized (26):

$$\text{lactate} \rightarrow \text{pyruvate} + 2H^+_{in} + 2e \quad (9)$$

Pyruvate is oxidized to acetate and CO_2, via acetyl coenzyme A and acetylphosphate intermediates. Energy conservation is achieved by substrate-level phosphorylation of ADP. The overall reaction is:

$$\text{pyruvate} + ADP + P_i \rightarrow \text{acetate} + CO_2 + ATP + 2H^+_{in} + 2e \quad (10)$$

The protons in reaction 10 are produced in the cytoplasm, and the electrons are accepted by cytoplasmic ferredoxin. The location and immediate fate of the protons and electrons produced in reaction 9 are uncertain, owing to the lack of knowledge of the topology of the membrane-bound lactate dehydrogenase. It has been assumed here, for simplicity, that the protons are cytoplasmic. Combining equations 1, 7, 9, and 10 for oxidation of 2 mol of lactate per mol of sulfate gives (59):

$$2\,\text{lactate} + SO_4^{2-} \rightarrow 2\,\text{acetate} + 2CO_2 + HS^- \quad (11)$$

while combination of equations 1, 7, and 10 gives:

$$4\,\text{pyruvate} + 2ADP + 2P_i + SO_4^{2-} \rightarrow 4\,\text{acetate} + 4CO_2 + 2ATP + HS^- \quad (12)$$

Contrary to reaction 3, reactions 11 and 12 do not lead to net proton translocation, because production and consumption of protons and electrons are not spatially separated. Use of lactate as the electron donor requires coupled, proton-driven ATP synthesis, since, contrary to growth on pyruvate, there is no net formation of ATP by substrate-level phosphorylation. To explain the generation of a coupled proton gradient, when lactate is used as the electron donor, Odom and Peck proposed that the electrons produced in reactions 9 and 10 cannot be used directly for sulfate reduction (44). Instead, these combine exclusively with protons in a reaction catalyzed by cytoplasmic hydrogenase:

$$8H^+_{in} + 8e \rightarrow 4H_2 \quad (13)$$

Cytoplasmic hydrogen then diffuses to the periplasm, where it is oxidized by periplasmic hydrogenases (reaction 2). This interesting proposal, known as the hydrogen cycling hypothesis, has been the subject of much debate. It requires the presence of both periplasmic and cytoplasmic hydrogenases in *Desulfovibrio* spp. Only the former has been found to be universally present. Formally, it does not differ from active proton transport, because the net effect of reactions 2 and 13 is the export of protons against their concentration gradient. In hydrogen cycling, the energy for this is supplied by the difference in redox potentials between participating cytoplasmic and periplasmic electrons. In addition to, or instead of, hydrogen cycling there may be other, as yet undiscovered, active proton transport mechanisms (e.g., involving the membrane-bound lactate dehydrogenase). If these pumps couple net transport of *m* protons, then reaction 11 changes to:

$$2\,\text{lactate} + SO_4^{2-} + mH^+_{in} \rightarrow HS^- + 2\,\text{acetate} + 2CO_2 + mH^+_{out} \quad (14)$$

Oxidation of pyruvate is similarly thought to be coupled to net proton translocation, and the import of these protons is coupled to synthesis of ATP, as has been discussed elsewhere (46).

A variety of short-chain alcohols and aldehydes can also serve as electron donors. NAD-dependent alcohol dehydrogenase from *Desulfovibrio gigas* has recently been purified and characterized (28), and the gene for aldehyde oxidase has been cloned and sequenced (58). A recent discovery was that the gram-negative sulfate reducer TD3 is able to use crude oil components (e.g., *n*-decane) as electron donors for sulfate reduction (54). This organism may contribute to the gradual increase in H_2S concentration (souring) that is often observed in oil fields over time as oil production by water injection progresses. Although TD3 is not a *Desulfovibrio* species, these can readily be isolated from oil field environments (69). The isolation and characterization of TD3 demonstrates that sulfate reducers can derive energy for growth from degradation of crude oil components. In addition to souring, sulfate reducers are feared in industrial settings for their ability to use metallic iron (Fe^0) as an electron donor for sulfate reduction (37, 43). Fe^0 is generated industrially by heating iron oxides in a reducing environment and reverts to its oxidized forms by corrosion processes. The use of Fe^0 as an electron donor for sulfate reduction by sulfate-reducing bacteria was first suggested in 1933 (64). Hydrogenases are thought to be important for the removal of electrons from the metal surface. The ubiquity of these enzymes, as well as other highly conductive redox proteins, such as cytochrome c_3, in the periplasm of *Desulfovibrio* spp. makes these organisms potentially excellent catalysts for this electron transport. Analysis of the microbial community present on corrosion coupons in oil field production waters indicated significant increases of selected *Desulfovibrio* species relative to the bulk fluid phase (69). *D. vulgaris* Marburg requires either lactate or hydrogen as an additional electron donor (12), indicating that this strain corrodes Fe^0 only cometabolically.

In addition to multiple electron donors, *Desulfovibrio* spp. can use electron acceptors other than sulfate. *Desulfovibrio desulfuricans* C4S can, like several other *D. desulfuricans* strains, reduce nitrate or nitrite to ammonia. This process is inhibited by 0.1 mM sulfide, whereas sulfate reduction is not

affected by sulfide concentrations of up to 5 mM (15). Nitrate reduction by *D. desulfuricans* C4S, which has a very high affinity for nitrate uptake (K_m = 0.05 mM), is thought to support the presence of large numbers of cells in rice paddy soil (15). Ironically, although Beyerinck already noted that working with pure strains is difficult unless strictly anaerobic conditions are employed (6), it appears that *Desulfovibrio* spp. can use oxygen as an electron acceptor under microaerophilic conditions (21). Oxidation of hydrogen proceeds with high respiration rates (250 nmol of O_2 min^{-1} mg of $protein^{-1}$) below 4% air. The process is cyanide insensitive, can be uncoupled, and yields ATP that can apparently be used for cell maintenance but not for growth, which still requires the absence of air. The periplasmic, monohemic cytochrome *c*-553 may be involved in this process by donating electrons to a membrane-bound oxidase that couples proton extrusion with transport of electrons to oxygen (33). A *Desulfovibrio*-like organism capable of reducing AsO_4^{3-} (arsenate) to AsO_3^{3-} (arsenite) was recently described (1). When grown with lactate as the electron donor for reduction of a mixture of arsenate and sulfate, it prefers arsenate. It can grow at staggeringly high concentrations (10 mM) of this toxic compound. Fe(III) has been suggested as an important electron acceptor for *Desulfovibrio* spp. in anaerobic sediments (11), although a pure strain capable of growth on media containing Fe(III) as the sole electron acceptor, as demonstrated for nitrate (15), has not yet been isolated. Sulfate reducers contribute to the reduction and immobilization of heavy metals, which is generally desirable except in the case of formation of the hazardous environmental pollutant methylmercury (10). Toxic Cr(VI) is reduced to the much less mobile Cr(III) (25, 38), while soluble U(VI) is reduced to insoluble U(III) (39). It is thought that periplasmic cytochrome c_3 serves as the metal reductase in all these cases (11, 38, 39). Sulfate reducers in constructed wetlands help to remove heavy metal ions from acid mine drainage effluents, and a variety of *Desulfovibrio* species can be isolated from such environments (57).

GENES INVOLVED IN ENERGY METABOLISM

The genus *Desulfovibrio* has a rather small genome. Initial estimates were 1.7 Mbp and 1.6 Mbp for the genomes of *D. vulgaris* and *D. gigas*, respectively (49). By pulsed-field electrophoresis of genomic digests with rarely cutting enzymes, a map and genome size of 2.1 Mbp were reported for *D. desulfuricans* G201 (71). Genes encoding proteins involved in energy metabolism were among the first to be cloned and sequenced, and the mechanism by which *Desulfovibrio* spp. derive energy from overall reaction 3, 5, or 8 is rapidly emerging from these studies (Fig. 1). *Desulfovibrio* species can have [Fe], [NiFe], and [NiFeSe] hydrogenases, which catalyze the reaction $H_2 \rightleftarrows 2H^+ + 2e$. A survey of genomes from 22 *Desulfovibrio* species indicated different distributions of these three enzymes (67). All species have the genes for [NiFe] hydrogenase, a heterodimer of a large (62-kDa) subunit and a small (28-kDa) subunit, which contains one active-site Ni ion, one [3Fe-4S] cluster, and two [4Fe-4S] clusters per mole of enzyme. The genes for periplasmic [Fe] hydrogenase and the [NiFeSe] hydrogenase are less universally distributed (67). The [NiFe] hydrogenase is a periplasmic enzyme that is also found in other gram-negative bacteria, e.g., in the genera *Escherichia*, *Azotobacter*, *Rhizobium*, and *Alcaligenes* (22, 65, 74). The [NiFe] hydrogenase from *Desulfovibrio* spp. is a soluble periplasmic enzyme, whereas the enzyme is anchored to the cytoplasmic membrane in these other bacteria. The fact that the *Desulfovibrio* enzyme exchanges electrons with a soluble periplasmic electron carrier,

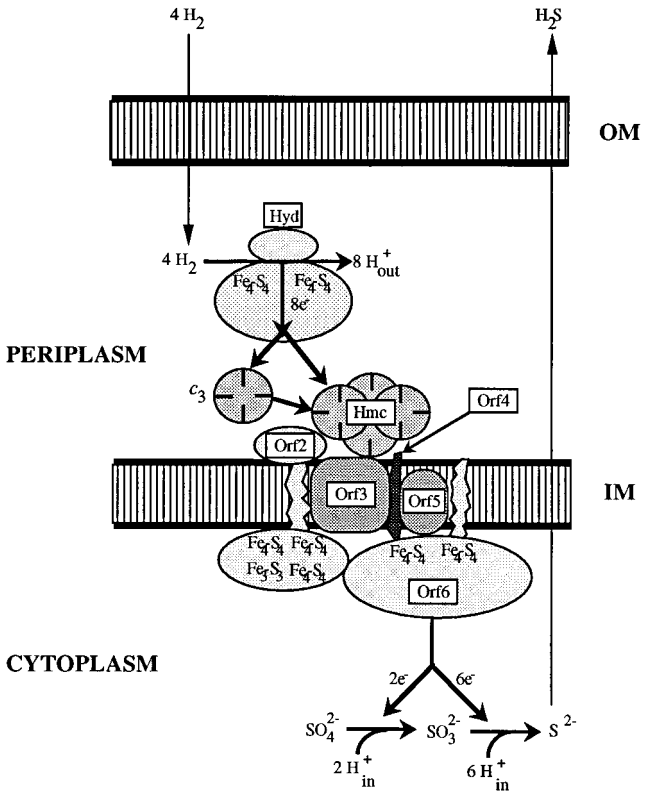

FIG. 1. Model for the functioning of the Hmc complex in the transport of electrons from hydrogen to sulfate. OM, IM, and Hyd are abbreviations for outer membrane, inner membrane, and hydrogenase, respectively. All other terms are defined in the text.

cytochrome c_3 (Fig. 1), while it donates electrons directly to a membrane-bound electron transport chain in these other genera explains the difference. A three-dimensional structure was reported for the [NiFe] hydrogenase from *D. gigas* (63), and determination of the structure of the enzyme from *D. vulgaris* Miyazaki is nearing completion (29). The *D. gigas* structure shows that the active-site nickel is liganded by conserved cysteine residues together with a second, as yet unidentified, metal ion. The structure also suggests paths for transfer of protons and electrons connecting the buried active site with the enzyme surface. The [NiFe] hydrogenase is exported to the periplasm with the help of a complex signal peptide at the N terminus of the small subunit (42). The [Fe] hydrogenase, a heterodimer of 46- and 9.6-kDa subunits, has a similar signal peptide at the N terminus of its small subunit. The small-subunit signal peptide is essentially the only region of significant sequence similarity between periplasmic [NiFe] and [Fe] hydrogenases, indicating that both enzymes are exported by a similar, but presently unknown, mechanism. The reason why some *Desulfovibrio* species have both a periplasmic [NiFe] and a periplasmic [Fe] hydrogenase is presently unclear. Peck has suggested that, of the eight electrons generated in reaction 2, two are donated by [Fe] hydrogenase to APS reductase for the reduction of APS to sulfite whereas six are donated by [NiFe] hydrogenase to sulfite reductase for reduction of sulfite to sulfide (46). He attributes hydrogen evolution (reaction 13) to [NiFeSe] hydrogenase. A universal proposal for the roles of these hydrogenases is difficult to defend because they are not universally distributed (67); e.g., *D. gigas* appears to contain only the periplasmic [NiFe] hydrogenase and lacks both the

[Fe] and [NiFeSe] hydrogenases. Thus, there must be multiple solutions to address the bioenergetic problems outlined in reactions 1 to 13. A specific role for [Fe] hydrogenase for growth on lactate-sulfate medium has been proposed (62), based on the observation that reduction of this enzyme by antisense RNA expression slows down growth on this medium considerably.

We assume, therefore, that when growing chemolithotrophically (reaction 3, 5, or 8), *Desulfovibrio* spp. catalyze hydrogen uptake through one or several periplasmic hydrogenases. Cytochrome c_3, a periplasmic, tetrahemic *c*-type cytochrome of known structure (13, 41), the gene for which has been cloned (66), can serve as the electron acceptor. Cytochrome c_3 is the most abundant cytochrome in *Desulfovibrio* spp. in terms of heme molarity. It displays very efficient intra- and intermolecular electron transport. Films of purified cytochrome c_3 are highly electron conducting (32), and we may attribute similar properties to the *Desulfovibrio* periplasm, which may explain the varied metal-reducing properties of the genus. However, despite its abundance, cytochrome c_3 cannot serve directly as the electron donor for sulfate reduction, because of its periplasmic location. The problem of how electrons are transported from the periplasmic hydrogenase-cytochrome c_3 system to the cytoplasmic sulfate reduction pathway was solved, in principle, with the discovery of the *hmc* operon in *D. vulgaris* Hildenborough (47, 52). This operon encodes six proteins: Hmc (55 kDa), Orf2 (40 kDa), Orf3 (43.2 kDa), Orf4 (6 kDa), Orf5 (25 kDa), and Orf6 (53 kDa). Hmc is a periplasmic cytochrome with 16 *c*-type hemes, which are present in cytochrome c_3-like domains. This protein has been purified and studied extensively by biophysical methods (8). The suggestion that each of the multiple cytochrome c_3 domains of Hmc may interact with a specific hydrogenase (52) is refuted by the recent isolation of Hmc from *D. gigas*, which has only the periplasmic [NiFe] hydrogenase (9). Orf2 is a transmembrane redox protein with four iron-sulfur clusters based on sequence similarity with DmsB, the membrane-bound electron-transferring subunit of dimethyl sulfoxide reductase of *Escherichia coli* (7). Orf3, Orf4, and Orf5 are highly hydrophobic integral membrane proteins with limited sequence similarity to integral membrane protein subunits of mitochondrial electron transport complexes, whereas Orf6 is thought to be a cytoplasmic protein with iron-sulfur clusters as prosthetic groups for electron transport. The suggested functions of Orf2 to Orf6 are currently being experimentally verified. The transmembrane nature of the redox protein complex encoded by the *hmc* operon (the Hmc complex) suggests the following steps in the reduction of sulfate by hydrogen (Fig. 1). (i) From hydrogenase, electrons are transferred either to cytochrome c_3 and then to the cytochrome c_3 domains of Hmc or directly to the latter (9). (ii) Electron flow from the periplasmic *c*-type hemes through the membrane-bound components Orf3 to Orf5 reduces the iron-sulfur clusters of the cytoplasmic Orf6 and/or Orf2. (iii) Orf6 and/or Orf2 transfers electrons to the cytoplasmic enzymes that function in sulfate reduction, APS reductase and dissimilatory sulfite reductase, either directly or through mediation of low-molecular-weight, cytoplasmic electron carriers, e.g., flavodoxin (35).

The Hmc complex is thus thought to link periplasmic and cytoplasmic electron transfer reactions (reactions 2 and 1, respectively). Overall reaction 3 or 5 applies depending on whether electron transfer by the Hmc complex is associated with active proton extrusion. The hydrogen cycling model would be validated if it could be shown that APS reductase and sulfite reductase can accept electrons only from the Hmc complex or other comparable transmembrane redox protein complexes (e.g., lactate dehydrogenase) and not from the cytoplasmic redox reactions 9 and 10.

Sequencing of the genes for the terminal dissimilatory sulfite reductase of *D. vulgaris* (31) has indicated a high degree of sequence similarity with the enzyme from *Archaeoglobus fulgidus*, a thermophilic sulfate-reducing archaebacterium (14). Dissimilatory sulfite reductase is a tetramer of two α (50-kDa) and two β (40-kDa) subunits, and both show approximately 60% sequence identity at the amino acid level. Sulfite reductase isolated from *D. vulgaris* appears also to have a smaller 11-kDa protein bound to it (30) which, in *D. desulfuricans* Essex 6, confers thiosulfate and trithionate reduction activity (55a). Considering the large phylogenetic distance between *D. vulgaris* and *A. fulgidus*, the observed conservation of α and β subunit sequences may mean that dissimilatory sulfite reductase in all sulfate-reducing bacteria is highly conserved. Thus, the enzyme responsible for the unbearable hydrogen sulfide evolution from Dutch city canals in summertime, reported by Beyerinck a century ago (6), has now been characterized at the sequence level. Its central function in a variety of environmental processes in which hydrogen sulfide production is important (metal corrosion, oil field souring, and heavy metal bioremediation) makes the determination of its three-dimensional structure an important future research target.

DIRECTED MUTAGENESIS OF *DESULFOVIBRIO* SPP.

Testing of ideas on the function of isolated genes in *Desulfovibrio* spp. (Fig. 1) requires a directed mutagenesis system. This is especially important in a microorganism that harbors so many redox proteins. Pairs of these can often be made to exchange electrons in vitro, but the significance of such exchanges for electron transport in *Desulfovibrio* spp. often remains obscure. Broad-host-range vectors of incompatibility group IncQ have been introduced into *Desulfovibrio* spp. by conjugation (3, 50, 61, 68). Genes for *Desulfovibrio* redox proteins incorporated in these plasmids were found to be considerably overexpressed, facilitating purification and subsequent biophysical study of [Fe] hydrogenase (61), cytochrome c_3 (68), hexadecaheme cytochrome (8), assimilatory sulfite reductase (56), and prismane protein (60). *Desulfovibrio* spp. provide a unique environment (low redox potential and high sulfide concentration) that may be advantageous to solve some common expression problems (disulfide scrambling, for instance). Its application as a host for high-level expression of foreign genes is now entirely feasible but has not yet been explored. Directed gene deletion has been reported by Rousset et al. (53), who managed to replace the genes for [NiFe] hydrogenase from *Desulfovibrio fructosovorans* with a kanamycin resistance cassette by electroporation of a nonreplicating plasmid. The mutant had 10% residual hydrogenase activity, owing to the presence of an [Fe] hydrogenase, which has subsequently been cloned and sequenced (40) and found to be quite different from the enzyme in *D. vulgaris* Hildenborough. The [Fe] hydrogenase of *D. fructosovorans* is cytoplasmic and has some similarity to the cytoplasmic NAD-reducing hydrogenase of *Alcaligenes eutrophus* (22). Although the mutant could still grow chemolithotrophically (reaction 8), the reduced growth rate and observed lag phase indicated a definite role for [NiFe] hydrogenase as a hydrogen uptake enzyme in *D. fructosovorans*. Mutagenesis of *D. vulgaris* Hildenborough could not be achieved through electroporation. However, mutagenesis by introduction of suicide plasmids through conjugation has recently been achieved (23), and this approach is now available to test the physiological function of genes that have been cloned and sequenced for this species. Characterization of an

indigenous plasmid of *D. desulfuricans* G200 has allowed the construction of a shuttle vector (70), and the isolation and characterization of two bacteriophages from *D. vulgaris* Hildenborough (55) may provide other ways to efficiently genetically manipulate *Desulfovibrio* spp. in the future.

DESULFOVIBRIO CHEMOTAXIS

Sequencing of 16S rRNA genes for phylogenetic analysis (see below) has led to definition of probes for microbial identification. Fluorescent versions of these probes (2) have been used to correlate the distribution of *Desulfovibrio* spp. and other sulfate reducers in biofilms with gradients in oxygen and total sulfide concentrations (51). Because the transition from an oxic to an anoxic environment occurs over short distances in these systems (51) that can be rapidly bridged by motile bacteria the question arises how motile *Desulfovibrio* spp. monitor their environment. Signal transduction in bacterial chemotaxis is initiated by transmembrane chemoreceptors, the methyl-accepting chemotaxis proteins (Mcps) (45). *E. coli* has four Mcps that mediate taxis towards attractants (sugars and amino acids) and away from repellents (organic acids). *D. vulgaris* Hildenborough has a much larger number of these Mcp-like chemoreceptor proteins: 12 genes (*dcrA* to *dcrL*) have been isolated from an ordered library of the *D. vulgaris* genome (16). The total number of *Desulfovibrio* chemoreceptor (*dcr*) genes has been estimated to be sixteen. The *dcr* gene family occupies an estimated 2% of the *D. vulgaris* genome, indicating that environmental sensing must be of vital importance to *Desulfovibrio* spp. One of these chemoreceptors (DcrA), encoded by the *dcrA* gene, has been characterized in detail. DcrA is a 73-kDa transmembrane protein with a topology very similar to that of the *E. coli* Mcps (17). Its N-terminal sensing domain contains a covalently bound *c*-type heme. Addition of oxygen or dithionite, which is expected to oxidize or reduce the heme iron, leads to changes in methylation of the cytoplasmic signal transduction domain (24). Thus, DcrA may, together with other members of the Dcr sensor protein family, help *Desulfovibrio* spp. to sense the oxygen concentration or redox potential of the environment and maintain the organisms in their optimal ecological niche.

DIVERSITY OF *DESULFOVIBRIO* SPP. IN THE ENVIRONMENT

Extensive microbiological analysis (72, 73) and 16S rRNA sequencing (18) have indicated that the genus *Desulfovibrio* is but one of eight different groups of sulfate-reducing eubacteria that can be isolated from the environment. Seven of these groups are gram negative, while one represents the gram-positive bacteria (*Desulfotomaculum*). The gram-negative, incompletely oxidizing (reactions 11 and 12), non-fatty acid-degrading *Desulfovibrio* spp. form group 1 in this taxonomic scheme. A more extensive comparison of 16S rRNA sequences with focus on group 1 members has revealed two branches (19), both in the δ subdivision of the proteobacteria. One branch contained five deep lineages of *Desulfovibrio* and *Desulfomicrobium* spp. The degree of 16S rRNA sequence similarity and percentage of DNA relatedness between these lineages were below the minimum genus level. A single new family name, *Desulfovibrionaceae*, was proposed for all species (either *Desulfovibrio* or *Desulfomicrobium* spp.) in these five lineages. The second branch contained misclassified "*Desulfovibrio*" spp. and other genera of sulfate-reducing bacteria (e.g., *Desulfobulbus*, *Desulfobacter*, and *Desulfococcus* spp.). These studies firmly established the wide diversity of *Desulfovibrio* species present in the environment and allowed the design of genus (e.g., *Desulfobacter*)- or species-specific probes. Sulfate reducers thrive in marine systems, owing to the high concentration of sulfate in seawater (28 mM). A large diversity of sulfate reducers in a marine sediment (2 to 3 cm beneath the sediment surface, where the rate of sulfate reduction was highest) was demonstrated by PCR amplification of extracted sediment DNA (20). Primers designed to amplify 16S rRNA genes of sulfate-reducing eubacteria (2) were used in this study. The diversity within the family *Desulfovibrionaceae* also follows from the low degree of cross hybridization of genomic DNAs in dot blot assays under stringent conditions. This allowed genomic DNA to be used as a probe for the presence of a species in the environment. Use of a reverse genome probe procedure allowed rapid screening of enrichment cultures against known isolates (69). These studies have indicated that *Desulfovibrio* spp. present in different environments (oil field production waters, acid mine drainage waters, sewage, and soil) are genomically distinct (57). Thus, the answer to the question posed by Beyerinck a century ago, whether the "sulfidferment" isolated from city canals is the same as that isolated from the seashore or from soil, is almost certainly negative. His query with respect to the distribution of *Desulfovibrio* spp. as a function of depth is now being addressed in the emerging field of subsurface microbiology. Culture and nucleic acid probe techniques should reveal details of changes in microbial populations, including sulfate-reducing bacteria, in the near future.

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