Sulfur-oxidizing epsilonproteobacteria are common in a variety of sulfidogenic environments. These autotrophic and mixotrophic sulfur-oxidizing bacteria are believed to contribute substantially to the oxidative portion of the global sulfur cycle. In order to better understand the ecology and roles of sulfur-oxidizing epsilonproteobacteria, in particular those of the widespread genus Sulfurimonas, in biogeochemical cycles, the genome of Sulfurimonas denitrificans DSM1251 was sequenced. This genome has many features, including a larger size (2.2 Mbp), that suggest a greater degree of metabolic versatility or responsiveness to the environment than seen for most of the other sequenced epsilonproteobacteria. A branched electron transport chain is apparent, with genes encoding complexes for the oxidation of hydrogen, reduced sulfur compounds, and formate and the reduction of nitrate and oxygen. Genes are present for a complete, autotrophic reductive citric acid cycle. Many genes are present that could facilitate growth in the spatially and temporally heterogeneous sediment habitat from where Sulfurimonas denitrificans was originally isolated. Many resistance-nodulation-development family transporter genes (10 total) are present; of these, several are predicted to encode heavy metal efflux transporters. An elaborate arsenal of sensory and regulatory protein-encoding genes is in place, as are genes necessary to prevent and respond to oxidative stress.

Only recently have epsilonproteobacteria been recognized as an environmentally relevant group of bacteria, as 16S rRNA-based sequencing surveys have identified them in a vast array of habitats, including brackish, marine, and subsurface (see, e.g., references 3, 11, 25, 39, 45, 71, and 74; reviewed in reference 6). Over the last years, quite a few cultured representatives of this group have been obtained from these environments, and currently most cultured members of the free-living epsilonproteobacteria are chemolithoautotrophs or mixotrophs, capable of either oxidizing reduced sulfur compounds and hydrogen with oxygen and/or nitrate or oxidizing hydrogen with elemental sulfur coupled to the fixation of inorganic carbon (reviewed in reference 6). These organisms use the reductive citric acid cycle for carbon fixation (24, 67). Given their abundance, sulfur-oxidizing epsilonproteobacteria, in particular members of the genus Sulfurimonas, are believed to be relevant to the function of the global sulfur cycle (6).

Genome data from these organisms would be key to metagenomic sequencing efforts in habitats where they are abundant and would also, by comparison to other epsilonproteobacteria, be helpful for determining the traits unique to a free-living, autotrophic lifestyle versus a host-associated, heterotrophic lifestyle. Recently, the genome sequences of Sulfurovum sp. strain NBC37-1 and Nitratireductor sp. strain SB155-2, two sulfur-oxidizing epsilonproteobacteria from deep-sea hydrothermal vents, were published; these sequences revealed that these organisms share many features with their pathogenic (e.g., Campylobacter and Helicobacter spp.) epsilonproteobacterial relatives (41). Given the remarkable variety of habitats where sulfur-oxidizing epsilonproteobacteria are found, it was of great interest to also conduct these analyses on nonvenom epsilonproteobacteria. To represent the abundant sulfur-oxidizing epsilonproteobacteria present in coastal marine sediments, we chose to sequence and analyze the genome of the sulfur-oxidizing chemolithoautotroph Sulfurimona-
including deep-sea hydrothermal vents, the oxic-anoxic interface marine sediments, bacteria belonging to this genus have been experimentally characterized, will not be predicted by this methodology. Dependent on comparison with known experimentally characterized transport-strate predictions, e.g., serine transport rather than transport of another amino and predicting approximate substrate specificity. However, making precise sub- transporter annotation pipeline that uses several predictive approaches such as phylogenetic operon, which may explain why, unlike close relatives (25, 50, 51, and 57). The main limitation of this approach is the ability to accurately predict precise transporter specificities. Based on both internal and external quality problems were addressed by sequencing finishing reads, and PHRED/PHRAP/CONSED were used for assembly (12, 13, 20). Automated and manual annotations were conducted by ORNL in a manner similar to that described previously (7, 57). Results were collated and presented via GenDB (37) for manipulation. Identification of genes encoding signal transduction and regulatory proteins. The complements of genes that encode signal transduction and regulatory pro- teins were compared among S. denitrificans DSM1251, *Thiomicrospira cramogena* XCL-2, and *Nitrooccus oceanus* ATCC 19707. To compare signal transduction and regulatory protein genes among these obligate autotroph genomes, genes had been generated using consistent methodology. For *Sulfurovum* sp. strain NBC37-1 and *Nitratiruptor* sp. strain SB155-2, data were collected from reference 41, for which slightly different methodologies were used to identify coding sequences (CDS). For *H. pylori*, the 16S gene is not collocated with the 23S and 5S genes in an operon. Additionally, an orphan 5S sequence is found in strain 26695.

**TABLE 1. Comparative genome features of epsilonproteobacteria**

<table>
<thead>
<tr>
<th>Species</th>
<th>Size (Mbp)</th>
<th>% Coding</th>
<th>% GC</th>
<th>rRNA operons</th>
<th>No. of CDS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sulfurimonas denitrificans</em> DSM1251</td>
<td>2.20</td>
<td>93.8</td>
<td>34.5</td>
<td>4</td>
<td>2,104</td>
</tr>
<tr>
<td><em>Sulfurovum</em> sp. strain NBC37-1</td>
<td>2.56</td>
<td>90.1</td>
<td>43.8</td>
<td>3</td>
<td>2,466</td>
</tr>
<tr>
<td><em>Nitratiruptor</em> sp. strain SB155-2</td>
<td>1.88</td>
<td>95.1</td>
<td>39.7</td>
<td>3</td>
<td>1,857</td>
</tr>
<tr>
<td><em>Campylobacter fetus</em> 82-40</td>
<td>1.80</td>
<td>90.0</td>
<td>33.3</td>
<td>3</td>
<td>1,719</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em> NCTC 11168</td>
<td>1.64</td>
<td>95.4</td>
<td>30.6</td>
<td>3</td>
<td>1,629</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em> RM1221</td>
<td>1.78</td>
<td>91.8</td>
<td>30.3</td>
<td>3</td>
<td>1,838</td>
</tr>
<tr>
<td><em>Helicobacter hepaticus</em> ATCC 51449</td>
<td>1.80</td>
<td>93.4</td>
<td>35.9</td>
<td>1</td>
<td>1,875</td>
</tr>
<tr>
<td><em>Helicobacter acinonyxis</em> Sheeba</td>
<td>1.55</td>
<td>89.0</td>
<td>38.2</td>
<td>2</td>
<td>1,618</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em> 26695</td>
<td>1.67</td>
<td>90.0</td>
<td>38.9</td>
<td>2</td>
<td>1,576</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em> 399</td>
<td>1.64</td>
<td>90.7</td>
<td>39.2</td>
<td>2</td>
<td>1,491</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em> HPAG1</td>
<td>1.59</td>
<td>91.0</td>
<td>39.1</td>
<td>2</td>
<td>1,544</td>
</tr>
<tr>
<td><em>Wolinella succinogenes</em> DSM1740</td>
<td>2.11</td>
<td>94.5</td>
<td>48.5</td>
<td>3</td>
<td>2,043</td>
</tr>
</tbody>
</table>

a Data for all taxa, except for *Sulfurovum* sp. strain NBC37-1 and *Nitratiruptor* sp. strain SB155-2, were collated from the Integrated Microbial Genomes webpage and had been generated using consistent methodology. For *Sulfurovum* sp. strain NBC37-1 and *Nitratiruptor* sp. strain SB155-2, data were collected from reference 41, for which slightly different methodologies were used to identify coding sequences (CDS).

b For *H. pylori*, the 16S gene is not collocated with the 23S and 5S genes in an operon. Additionally, an orphan 5S sequence is found in strain 26695.

**RESULTS AND DISCUSSION**

**Genome structure.** The *S. denitrificans* DSM1251 genome is one of the largest epsilonproteobacterial genomes yet sequenced, consisting of a single 2.2-Mbp chromosome (Table 1). The coding density and G+C content are similar to those of the other epsilonproteobacteria (Table 1). Four rRNA operons are present, which, due to their elevated G+C content (~50%) relative to the genome average (34.5%), are visible as positive G+C content anomalies on the genome map (Fig. 1). Three of these operons (the 16S-rRNA^T^rRNA^C^rRNA^S^1S-SS operons) are 100% identical and are oriented in the same direction, while the fourth (the 16S-rRNA^T^rRNA^C^rRNA^S^2S-SS operon) is in the opposite orientation, and its 5S and 23S genes each have a single nucleotide substitution compared to the others. The free-living *S. denitrificans*, *Sulfurovum* sp. strain NBC37-1, and *Nitratiruptor* sp. strain SB155-2 have more rRNA operons than those epsilonproteobacteria that are known to be exclusively host related (Table 1), which is likely a reflection of an adaptation to fluctuation environmental conditions and the necessity for versatility (30, 41, 65).

Two large (17,627-bp) identical transposons are apparent as negative-G+C-content anomalies (30.0%) (Fig. 1). Flanked by identical 12-bp inverted repeats, these transposons (Suden_0690 to Suden_0702 and Suden_1587 to Suden_1599) include genes encoding transposases as well as proteins similar to the Tn1B (46%) and TnIQ (47%) transposase accessory proteins found in mercury resistance transposons in *Xanthomonas* sp. strain W17 and other systems (29). These transposons also include genes encoding a type I restriction-modification methyltransferase and restriction enzyme (see the supplemental material). Interestingly, one of the copies of this transposon interrupts a flagellin biosynthetic operon, which may explain why, unlike close relatives (25,
family genes (10 genes [Suden_0270, Suden_0536, Suden_0799, Suden_0876, Suden_0877, Suden_0883, Suden_1281, Suden_1440, Suden_1449, and Suden_2011]) compared to what is seen for other epsilonproteobacteria, including the two hydrothermal vent species (2 to 6 genes). Many of these are predicted to encode transporters for metal efflux in *S. denitrificans*. As in *W. succinogenes* and the two hydrothermal vent epsilonproteobacteria, an apparent operon that encodes a cytoplasmic arsenate reductase (Suden_0314), arsenite permease (Suden_0313), and regulatory protein ArsR (Suden_0315) is present (41, 61). Apparently, the sediment ecosystem inhabited by *S. denitrificans* requires a level of resistance to metals and other toxins similar to or perhaps enhanced over that of the digestive tract habitats and hydrothermal vents favored by the other sequenced species.

**Electron donors.** *S. denitrificans* was originally isolated in a chemostat with thiosulfate as the electron donor and nitrate as the electron acceptor (70). However, prior to this study, the pathways and complexes involved were not identified. Neutrophilic sulfur-oxidizing bacteria use two types of sulfur oxidation pathways: one involving a multienzyme complex catalyzing the complete oxidation of reduced sulfur compounds to sulfate (Sox pathway) (15, 28), and another implementing sulfite and elemental sulfur as important intermediates (27, 47, 59). The genome of *S. denitrificans* reveals that the oxidation of reduced sulfur compounds proceeds via the Sox pathway (Fig. 2). Homologs for genes encoding all components that are required for a fully functional complex in vitro, i.e., SoxB, SoxXA, SoxYZ, and SoxCD (15), could be identified. As for other obligate sequenced autotrophs (41, 57), the sox genes in *S. denitrificans* do not occur in one cluster, as in the model organism *Paracoccus pantotrophus* GB17 (15), but in different parts of the genome. *S. denitrificans* has basically two clusters, one containing soxXYZAB (Suden_0260 to Suden_0264) and another one containing soxZYCD (Suden_0257 to Suden_0260). SoxZY are known to interact with both SoxAB and SoxCD, and their duplication could possibly indicate differential regulation of these two loci. SoxCD has homologies to sulfite dehydrogenase (SorAB) but has been shown to act as a sulfur dehydrogenase (15). In addition, it has recently been shown that organisms that lack soxCD but do have soxB, soxX, and soxYZ use the Sox system to oxidize thiosulfate to sulfate, which is either stored inside the cell or excreted (21). However, elemental sulfur formation by *S. denitrificans* has not been reported. Recently, sulfur oxidation enzymes were also measured in the closely related bacteria *Sulfurimonas autotrophica* and *Sulfurimonas paralvinellae* (67). In this case, sulfite dehydrogenase was detected using an assay that would not be expected to measure such activity were these organisms to use the Sox system (C. G. Friedrich, personal communication), indicating that other *Sulfurimonas* spp. might either not use the Sox system or use a modified version of it. In this regard, it is interesting that the SoxC sequence identities of *S. denitrificans* to sequences of other organisms that have a contiguous sox gene set are significantly lower (44%) than when SoxC sequences from organisms in which sox genes occur in one cluster are compared among themselves (>63%). Both soxB and soxC genes exhibit the highest similarities with genes from *Sulfurovum* sp. strain NBC37-1 (41), which suggests that both clusters of sox genes are not recent additions to this epsilonproteobacterial lineage. In fact, a phylogenetic analysis based on a large number of SoxB sequences from a variety of sulfur-oxidizing bacteria is even

![FIG. 1. Map of the *Sulfurimonas denitrificans* DSM1251 genome. The two outer rings include protein-encoding genes, which are color coded based on their membership in COG categories. Ring 3 depicts the deviation from the average G+C content level (%), while the innermost ring is the GC skew ([G−C]/[G+C]). R1, R2, R3, and R4 are rRNA operons (with their orientations indicated with arrows), and the two regions marked T are identical large transposons. The G+C content level (%), while the innermost ring is the GC skew ([G−C]/[G+C]). R1, R2, R3, and R4 are rRNA operons (with their orientations indicated with arrows), and the two regions marked T are identical large transposons. The G+C and GC skew rings were calculated with a sliding window of 10,000 bp with a window step of 100. Cons., conserved.](image-url)
suggestive of an origin of the Sox system in epsilonproteobacteria (36).

Besides those for the Sox system, \textit{S. denitrificans} also has a gene encoding a sulfide:quinone oxidoreductase (Suden_0619). Sulfide:quinone oxidoreductase catalyzes the oxidation of sulfide to elemental sulfur in \textit{Rhodobacter capsulatus} (56), leading to the deposition of sulfur outside the cells. At present, its role in \textit{S. denitrificans} is unclear, as this species has not been shown to deposit elemental sulfur, though this possibility has not been exhaustively explored with differing cultivation conditions.

The genome also provided evidence for the ability to use H$_2$ and formate as electron donors (Fig. 2). Based on this information, \textit{S. denitrificans} was successfully cultivated with H$_2$ as its electron donor and nitrate as the electron acceptor (S. Sievert and S. Molyneaux, unpublished data). The \textit{S. denitrificans} genome encodes two Ni-Fe hydrogenase systems: one cytoplasmic enzyme and one membrane-bound hydrogenase complex. The genes encoding the two subunits of the cytoplasmic enzyme (which lack TAT motifs) (Suden_1437 and Suden_1438) are adjacent to genes encoding the periplasmic hydrogenase (its small subunit has a TAT motif, and a $b$-type cytochrome subunit would function to anchor it to the membrane and shunt electrons to the quinone pool) (Suden_1434 to Suden_1436). The small subunit of the cytoplasmic hydrogenase of \textit{S. denitrificans} forms a cluster with sequences from the two deep-sea hydrothermal vent epsilonproteobacteria and \textit{Aquifex aeolicus} and is distantly related to H$_2$-sensing hydrogenases of alphaproteobacteria and cyanobacteria (41). Nakagawa et al. (41) suggest that the cytoplasmic hydrogenase acts as an H$_2$-sensing hydrogenase in \textit{Sulfurovum} sp. strain NBC37-1 and \textit{Nitratiruptor} sp. strain SB155-2. However, an alternative, and in our view more likely, function for the cytoplasmic enzyme as a catalytically active hydrogenase is suggested by the sequence similarity of both subunits to the enzyme from \textit{A. aeolicus}. In \textit{A. aeolicus}, the cytoplasmic hydrogenase can reduce electron acceptors with very negative redox midpoint potentials and therefore has been suggested to provide low-potential electrons to the reductive citric acid cycle (4). This would circumvent the necessity for reverse electron transport and thus increase its growth efficiency, similar to what has been found for certain Knallgas bacteria using the Calvin cycle for CO$_2$ fixation. Further experiments are needed to confirm the actual
role of the cytoplasmic hydrogenase. Following these hydro- 
genase genes are several genes encoding hydogenase-as- 
semble related functions (Suden_1424 to Suden_1433).

A formate dehydrogenase complex is encoded by an operon 
similar in gene order to one found in W. succinogenes 
(Suden_0816 to Suden_0824). Formate dehydrogenase α sub-
units contain a selenocysteine residue (26) which is encoded 
by a stop codon. A putative selenocysteine codon (TGA) 
followed by a palindromic region was found between two open 
reading frames that are homologous to the amino and carboxy ends of 
formate dehydrogenase; accordingly, these open reading 
frames have been combined into a single coding sequence for 
the α subunit of this enzyme, Suden_0820, which includes the 
molybdopterin-binding and iron-sulfur cluster domains typi-
cally found in this subunit, as well as the TAT pathway signal 
sequence which would shunt this subunit to the periplasm. The 
β and γ subunits are encoded by Suden_0819 and Suden_0818, 
respectively, with the latter having an unprecedented N-termi-
mal addition with two more predicted transmembrane seg-
ments (making six in total). Suden_0824 encodes a ferredoxin, 
which may shuttle the electrons from formate oxidation to 
cellular processes. Formate dehydrogenase maturation is likely 
facilitated by the products of Suden_0823, which encodes a 
TorD family protein that functions in molybdoprotein forma-
tion, and Suden_0817, which encodes an FdhD/NarQ family 
member of the heme-copper oxidase (HCO) superfamily (16), 
of nitrous oxide-forming nitric oxide reductase (cNOR), a 
member of the heme-copper oxidase (HCO) superfamily (16), 
are usually clustered together with additional genes required 
for enzyme assembly and activation (76). These latter genes, 
norD and norQ, are missing from the S. denitrificans genome. 
While there is evidence for functional cNOR in bacteria that 
lack the norQ (cbbQ) gene, there are presently no experimen-
tal reports that demonstrate the functionality of cNOR in 
bacteria that also lack the norD gene. Attempts to test this for 
Hydrogenobacter thermophilus with Pseudomonas aeruginosa or 
Escherichia coli as expression hosts were inconclusive (66). The 
S. denitrificans norCB genes are most closely related by se-
quence similarity to the norCB genes in the genomes of H. 
thermophilus and Methylococcus capsulatus as well as Sulfu-
rovum sp. strain NBC37-1 and Nitratiruptor sp. strain SB155-2, 
the latter of which are two newly sequenced marine epilon-
proteobacteria (41). All of these genomes also lack the norD 
gene.

Even though the functionality of cNOR is questionable, S. 
denitrificans is a complete denitrifier and must be able to 
reduce NO. Attempts to find other inventory implicated in NO 
reduction were successful and yielded additional candidate 
systems. One of them, NADH:flavorubredoxin-NOR, also 
known as the NorVW complex (17, 19), was also not complete 
and thus likely nonfunctional because a NorW-encoding gene 
was not identified. Interestingly, the genome encodes NorV in 
the unusual form of two genes: one encoding a rubredoxin 
(Suden_1582) is succeeded by a flavodoxin gene (Suden_1581). 
Although both cNOR and NADH:flavorubredoxin-NOR may 
not have catalytic activity, it is possible that their NO-binding 
capacity has a function in NO sequestration and detoxification.

Interestingly, the S. denitrificans genome also encodes a pre-
viously unidentified member of the HCO superfamily that is 
also a candidate for catalyzing nitric oxide reduction. These 
HCO genes follow a set of pseudogenes normally involved in 
nitrate reduction (Suden_0100 to Suden_0102). Based on 
structural modeling and genome analysis, it is expected that 
this new HCO family is a novel nonelectrogenic quinone-oxi-
dizing nitric oxide reductase, gNOR (J. Hemp, M. G. Klotz, 
L. Y. Stein, and R. B. Gennis, unpublished data). The gNOR 
gene, encoded by the norGHJ genes (Suden_0103 to Suden_0105), 
is unique within the HCO superfamily in that it 
exhibits a novel active-site metal ligation, with one of the three 
conserved histidine ligands being replaced with an aspartate. 
This ligation pattern strongly suggests that the active-site metal 
is an iron. Structural modeling of members of the gNOR family 
has identified three conserved acidic residues which form a 
charged pocket within the active site, a feature shared with the 
cNOR family (49). Besides in S. denitrificans, gNOR also ap-
pears to be present in Sulfurovum sp. strain NBC37-1 and 
Persephonella marina strain EX-H1, whereas it is missing from 
Nitratiruptor sp. strain SB155-2. Since phylogenetic analysis 
demonstrates that nitric oxide reductase activity has evolved 
multiple times independently within the heme-copper super-
family, these shared features between the distantly related 
gNOR and cNOR families are interesting examples of conver-
genesis evolution (Hemp et al., unpublished).
Nitrous oxide reduction is carried out by nitrous oxide reductase encoded by an unusual nos gene cluster (Suden_1298 to Suden_1308) similar to one previously identified in *W. succinogenes* (63). As in *Wolinella*, the NosZ in *S. denitrificans* (Suden_1298) contains a C-terminal extension of about 200 residues that carries a monoheme cytochrome *c* binding motif (CXGCH), suggesting that it too functions as a cytochrome *c* nitrous oxide reductase (Fig. 2). This feature is also shared by NosZ of *Sulfurovum* sp. strain NBC37-1 and *Nitratisporus* sp. strain SB155-2, which form a cluster with NosZ from *S. denitrificans* (see Fig. S3 in the supplemental material), possibly allowing the design of primers to screen for the presence of denitrifying epsilonproteobacteria in the environment. It has been hypothesized that the nos gene cluster in *W. succinogenes* codes for proteins involved in an electron transport chain from menaquinol to cytochrome *c* nitrous oxide reductase (63), and it is likely that the same holds true for *S. denitrificans*, as well as *Sulfurovum* sp. strain NBC37-1 and *Nitratisporus* sp. strain SB155-2. In addition, *S. denitrificans* has an almost identical copy of nosZ next to a c553-type monoheme cytochrome *c* (Suden_1770, Suden_1769), but its function is at this point unknown. Interestingly, *S. denitrificans* also has a gene coding for a large subunit of a ferredoxin-nitrite reductase (*nirB*; Suden_1241), which could be involved in nitrite assimilation or detoxification (8). However, no gene coding for the small subunit was identified, raising questions about its function.

Additional electron acceptors are suggested by this organism’s gene complement (Fig. 2). Like most alpha-, beta-, gamma-, and epsilonproteobacteria but unlike deltaproteobacteria, the *S. denitrificans* genome contains a cluster of four genes that encode the FixNOQP proteins, which constitute a proton-consuming fumarate reductase, one of which has a subunit that would anchor it to the membrane (Suden_1028 to Suden_1030), while the other lacks this subunit and may be cytoplasmic (Suden_0037, Suden_0038). The membrane-bound form is unusual in that it has a cysteine-rich type-F membrane anchor. It is similar to SdhABE from *W. succinogenes*, which has been characterized as a membrane-bound fumarate-reducing complex with subunits SdhAB facing the periplasm. In contrast to *W. succinogenes*, the *S. denitrificans* gene encoding subunit A is about 43 residues shorter and lacks the TAT signal peptide present in its *W. succinogenes* homolog, something that appears to be shared with *Sulfurovum* sp. strain NBC37-1 and *Nitratisporus* sp. strain SB155-2, both of which are also chemolithoautotrophic epsilonproteobacteria using the reductive citric acid cycle for carbon fixation. Thus, it appears that in these organisms the membrane-bound fumarate-reducing complex faces into the cytoplasm. At present, the exact function of the two fumarate reductases is unknown, although it is likely that the membrane-bound one, due to its potential for additional energy generation, might be involved in the reductive citric acid cycle for autotrophic carbon fixation. The intriguing possibility that *S. denitrificans* might also be able to carry out fumarate respiration has to await further experimentation. However, *S. denitrificans* does not contain an *frdABC* operon typical for menaquinol:fumarate reductase sustaining fumarate respiration in other epsilonproteobacteria.

The acetyl coenzyme A (acetyl-CoA) and oxaloacetate produced by the reductive citric acid cycle could be funneled to central carbon metabolism: acetyl-CoA could be converted to pyruvate via pyruvate:acceptor oxidoreductase (see above) and oxaloacetate could be used to form phosphoenolpyruvate via phosphoenolpyruvate carboxykinase (Suden_1696). Acetyl-CoA could also be directed to fatty acid synthesis (acetyl-CoA carboxylase [Suden_1174, Suden_1608]). Genes that suggest an ability to supplement autotrophic growth with acetate assimilation are present in all three sulfur-oxidizing epsilonproteobacteria. Two possible systems for converting acetate to acetyl-CoA are present: acetyl-CoA ligase (Suden_1451), as
well as phosphate acetyltransferase (Suden_0055) and acetate kinase (Suden_0056). These are also present in *Sulfurovum* sp. strain NBC37-1, while acetate kinase is absent from *Nitratiruptor* sp. strain SB155-2. Perhaps the two systems have different affinities for acetate, as has been demonstrated for methanogens (60), and are differentially expressed depending on the environmental concentrations of this organic acid.

In order for *S. denitrificans* to grow autotrophically using the reductive citric acid cycle, there must be a means of carboxylating pyruvate to form oxaloacetate. In some organisms, this is accomplished by the tandem activities of phosphoenolpyruvate synthetase and phosphoenolpyruvate carboxylase, while others use pyruvate carboxylase (55). In contrast to the two autotrophic epsilonproteobacteria *Sulfurovum* sp. strain NBC37-1 and *Nitratiruptor* sp. strain SB155-2, which have genes encoding phosphoenolpyruvate synthetase as well as pyruvate kinase (41), *S. denitrificans* does not appear to have any genes that might encode an enzyme that could interconvert phosphoenolpyruvate and pyruvate. Instead, it may use pyruvate carboxylase, as genes encoding both the biotin carboxylase subunit (Suden_0622) and biotin carboxyl carrier subunit (Suden_1259) of this enzyme are present. The biotin carboxyl carrier subunit gene (Suden_1259) occurs in an apparent operon with other genes homologous to the subunits of sodium-transporting oxaloacetate decarboxylase (Suden_1258 to Suden_1260). Suden_1259, which encodes the α subunit of this complex, has a high level of sequence similarity with pyruvate carboxylase genes from various *Campylobacter* species (including one from *C. jejuni* which has been biochemically characterized) (72), while the β and γ subunits (Suden_1258 and Suden_1260), which are absent from the heterotrophic epsilonproteobacteria, are similar to those found from *Sulfurovum* sp. strain NBC37-1, *Nitratiruptor* sp. strain SB155-2, many gammaproteobacteria, many *Chlorobia* spp., and *Desulfotalea psychrophila*, a deltaproteobacterial sulfate reducer. Heterotrophic organisms that have this complex ferment citrate. After cleaving citrate to acetate and oxaloacetate, they use the oxaloacetate decarboxylase complex to couple the exothermic decarboxylation of this organic acid to the extrusion of sodium ions. For these organisms, the other genes necessary for citrate fermentation (e.g., the gene for the citrate transporter) are nearby (9). This is not the case for *S. denitrificans*. An alternative function for Suden_1259 is suggested by phylogenetic analysis, which places it within a clade with the biochemically characterized pyruvate carboxylase from *C. jejuni* (Fig. 4) and separate from biochemically characterized oxaloacetate decarboxylase genes from *Klebsiella pneumoniae* and *Vibrio cholerae* (5). Other members of this clade include the genes from *Chlorobia* spp., *Sulfurovum* sp. strain NBC37-1, and *Nitratiruptor* sp. strain SB155-2, which also use the reductive citric acid cycle for carbon fixation and are not known to ferment citrate. It is possible to operate the oxaloacetate decarboxylase complex as a pyruvate carboxylase by imposing a sodium gradient across the membrane (10). It is tempting to speculate that in the autotrophic epsilonproteobacteria, *Chlorobia* spp., and possibly *D. psychrophila*, this complex functions as a pyruvate carboxylase. Interestingly, the sequenced autotrophic epsilonproteobacteria and *Desulfotalea psychrophila* are all marine organisms, and *Chlorobia* spp. evolved in the marine environment (1). Only 5 of the 10 sequenced *Chlorobia* spp. have the sodium-transporting oxaloacetate decarboxylase/pyruvate carboxylase complex, while the other 5, including *C. tepidum*, have the α subunit (on which Fig. 4 is based) but not the three-
subunit pump. With the exception of *C. limicola*, which was isolated from a mineral hot spring, all *Chlorobia* spp. containing the sodium pump have a requirement for sodium. Thus, it is likely that these organisms have found a way to couple pyruvate carboxylation, which is energetically unfavorable, to a sodium gradient, something that was previously proposed but never shown for an organism (10). Whether the complex encoded by Suden_1258 to Suden_1260 functions as a pyruvate carboxylase or an oxaloacetate decarboxylase is a key point begging clarification, which will be nontrivial, given that a genetic system has not been developed for this organism.

Carbon fixed by the reductive citric acid cycle can be shunted through gluconeogenesis, as all genes necessary for this pathway are present. The presence of genes encoding citrate synthase (Suden_2100) and ATP-dependent (irreversible) phosphofructokinase (Suden_0549) are enigmatic, as their roles in this obligate autotroph are unclear.

Genes are apparent whose products could utilize the carbon skeletons synthesized by central carbon metabolism for ammonia assimilation (see below) and amino acid, nucleotide, fatty acid, and phospholipid synthesis. Cysteine biosynthesis is notable in that the reduction of sulfate proceeds via adenosine 5'-phosphosulfate rather than 3'-phosphoadenylylsulfate in a pathway that was until recently known only for plants (42). Genes encoding assimilatory sulfate reduction cooccur in an apparent operon (Suden_0154 to Suden_0160). Most likely, this operon-like structure is turned on or off depending on whether *S. denitrificans* is inhabiting an environment with a

---

**FIG. 4.** Phylogenetic relationships of α subunits of oxaloacetate decarboxylase (OAD), pyruvate carboxylase (PVC), oxoglutarate carboxylase (OGC), and type III pyruvate carboxylase to the product of Suden_1259 of *Sulfurimonas denitrificans*. Sequences were aligned using the program package MacVector. Neighbor-joining and parsimony trees based on the predicted amino acid sequences were calculated using PAUP 4.0b10. Bootstrap values (1,000 replicates) for the major nodes are given for the neighbor-joining (first value) and parsimony (second value) analyses. *P. aestuarii, Prosthecochloris aestuarii.*
high concentration of reduced inorganic sulfur compounds. Interestingly, sulfate assimilation in Sulfurovum sp. strain NBC37-1 and Nitratiruptor sp. strain SB155-2 appears to proceed via 3'-phosphoadenylylsulfate.

Nitrogen assimilation. The S. denitrificans genome contains nirC (focA) and nirB genes, encoding the formate-nitrite transporter (Suden_0716) and the large subunit of NAD(P)H-dependent ammonia-forming siroheme nitrite reductase (Suden_1241), respectively, along with the inventory for siroheme synthesis (Suden_1977, cysG, siroheme synthase; Suden_1989, cobA-cysG, uroporphyrinogen III methylase); however, it lacks the nirD gene, which encodes the small subunit of siroheme nitrite reductase. Because the genome also lacks nrfHA genes, which encode respiratory nitrite ammonification capacity in many delta- and epsilonproteobacteria (62), it appears that Sulfurimonas is solely dependent on ammonia uptake from the environment. The genome indeed contains two genes encoding different ammonia permeases (noted above) (22, 43), one AmtB-like (Suden_0641) and one rhizobial factor-like (Suden_0643) permease, which are clustered together with the gene encoding nitrogen regulatory protein PII (glnK, Suden_0642). Whereas AmtB proteins function as ammonia gas uptake channels, the substrate for Rh-like protein channels is still debated and includes ammonia as well as CO₂ (43). The genome contains also all the additional genes needed for 2-oxoglutarate sensing and the regulation of nitrogen assimilation (reviewed in reference 34).

Chemotaxis and other regulatory and signaling proteins. Close relatives of S. denitrificans are motile, while this particular strain is nonmotile, probably due to the interruption of a flagellar biosynthetic operon by a transposon (see “Genome structure” above). Based on the presence of all of the genes necessary to encode the flagellar apparatus, none of which display any evidence of degeneration, an abundant sensory apparatus necessary to detect the presence of chemotacticants or repellants and communicate this information to the flagellar motor, as well as the sequence identity of this transposon with a duplicate in the genome (see above), it is likely that nonmotility is a recently acquired property. Interestingly, many of the genes encoding the chemotaxis components are in a large cluster with multiple kinases and response regulators (Fig. 5), as in Nitratiruptor sp. strain SB155-2 (41), suggesting interconnectivity between the chemotaxis and other signal transduction systems. Perhaps the original enrichment and isolation procedure for this strain (in a chemostat) might have selected for a nonmotile strain.

The S. denitrificans genome encodes a relative abundance of signaling proteins. Particularly well represented in these genomes are genes encoding proteins with EAL and GGDEF domains (based on PFAM hits, 16 and 38 genes, respectively), which likely function in the synthesis and hydrolysis of the intracellular signaling compound cyclic diguanulate (53). Further, six proteins with PAS/PAC domains which may function as redox sensors are encoded (75). The genomic repertoire of signaling and regulatory genes was compared with those of two other free-living, obligate chemolithoautotrophs for which these data are available (Table 2). Some features are similar to Thiomicrospira crunogena: both of these species have a relative abundance of signal transduction proteins compared to Ni-

![FIG. 5. A large gene cluster from the Sulfurimonas denitrificans genome that includes many of the genes for chemotaxis signal transduction.](image-url)
trosococcus oceani (31), which may be a response to habitats spatially (sediments; S. denitrificans) or temporally (hydrothermal vents; T. crunogena) more heterogeneous than the open ocean (N. oceanii). Both have a large number of genes encoding methyl-accepting chemotaxis proteins (Table 2). Unlike what is seen for T. crunogena, none of the methyl-accepting chemotaxis proteins from S. denitrificans are predicted to have PAS/PAC domains that could bind redox-sensitive cofactors (57), but a gene encoding a protein with a PAS/PAC domain is present in the large cluster of chemotaxis genes described above (Fig. 5), suggesting potential communication between sensing cellular or environmental redox conditions and the chemotactic apparatus. Another similarity between T. crunogena and S. denitrificans is an abundance of genes predicted to be involved with cyclic nucleotide signal transduction, and many of these are predicted to have EAL and/or GGDEF domains, indicating a role for cyclic diguanylate in intracellular signaling in this organism. Many of these predicted proteins also have PAS/PAC domains, as in T. crunogena (57).

Unique among the three species compared here, S. denitrificans has a relative abundance of signal transduction histidine kinases and an expanded complement of winged-helix family two-component transcriptional regulators (Table 2). Both T. crunogena and N. oceanii can use a rather limited variety of electron donors and acceptors compared to S. denitrificans. Perhaps this expansion in histidine kinases and transcriptional regulators coordinates the expression of the complexes necessary for the oxidation of multiple electron donors (e.g., H₂, reduced sulfur compounds, formate) and the reduction of multiple electron acceptors (O₂ and NO₃).

Oxidative stress. S. denitrificans has reasonably elaborate defenses against oxidative stress, on par with what has been observed for the pathogenic epsilonproteobacterium H. pylori (73). Initially, this was considered surprising to us, because this nonpathogenic species does not have to elude the oxidative arsenal of a host immune system, nor, as a microaerophile capable of growth via denitrification, does it grow in the presence of high concentrations of oxygen. However, given the presence of enzymes with labile iron-sulfur clusters with irreplaceable roles in central carbon metabolism in this organism (e.g., pyruvate:acceptor oxidoreductase [Suden_0096 to Suden_0099], 2-oxoglutarate:acceptor oxidoreductase [Suden_1053 to Suden_1055]), perhaps added defenses are a necessary part of survival.

Several genes whose products could prevent the buildup of intracellular Fe²⁺ that can spur hydroxyl radical generation via the Fenton reaction are present. A gene that encodes the Fur protein (Suden_1272), which regulates iron uptake (14), is present. A gene encoding iron-binding ferretin (Suden_1760) is also present.

Many enzymes to detoxify reactive oxygen and nitrogen species are encoded in this genome. An iron/manganese superoxide dismutase (Suden_1129) is present in this species but not in the other sulfur-oxidizing epsilonproteobacteria (41). This enzyme and ruberythrin (Suden_0739) could convert superoxide to hydrogen peroxide, which in turn could be dispatched by catalase (Suden_1323), peroxiredoxins (Suden_0132, Suden_0630, Suden_1778, Suden_1803), or cytochrome c peroxidase (Suden_0214, Suden_1585). Peroxiredoxins, particularly alkylhydroperoxide reductases, might be specifically targeted toward low levels of H₂O₂ or organic peroxides (58, 73). To dispense with nitric oxide that escapes the periplasmic and membrane-associated respiratory nitrogen reduction complexes, a truncated hemoglobin gene (Suden_0993) which may convert nitric oxide to nitrate is present (46). Thioredoxins (Suden_0342, Suden_0501, Suden_1867, Suden_2099) and thioredoxin reductase (Suden_1869) could funnel electrons to these oxidative stress proteins, as glutathione does not appear to play this role in this organism, since genes encoding glutathione synthetase or gamma-glutamyl-cysteine ligase are absent.

Genes are apparent whose products could enable a cell to cope with damage inflicted by any reactive oxygen or nitrogen species that escape cellular defenses. Endonucleases III (Suden_0516) and IV (Suden_1835) and MutS (Suden_0755) could repair oxidative DNA damage. Methionine sulfoxide reductase (Suden_0012) and alkylhydroxide reductase (Suden_1778) could contend with any methionine residues or lipids that had been oxidized by interaction with reactive oxygen or nitrogen species, while nitroreductases (Suden_0519, Suden_1158) could prevent oxidized cellular nitrogenous and other compounds from generating peroxide.

Conclusions. S. denitrificans has several unique features which differentiate it from the other epsilonproteobacteria that have been sequenced to date. It has a larger genome than most of the others, which likely provides the sensory, regulatory, and metabolic versatility necessary for survival in a habitat more heterogeneous than found in a metazoan host. For example, the numerous genes whose products have redox sensory domains likely function to position these cells in the redoxcline to enable them to obtain the electron donors and acceptors needed for growth. Furthermore, these cells are quite versatile with respect to electron donors and acceptors, as the genome data suggest a capability of using donors and acceptors beyond those based on cultivation studies. Although S. denitrificans has been isolated from coastal marine sediments, its genome shares many features with two recently described autotrophic deep-sea hydrothermal vent epsilonproteobacteria, including the potential to utilize a variety of redox substrates (hydrogen gas, reduced sulfur compounds, oxygen, and nitrate), its responses to oxidative stress and high metal content, and a genome size intermediate between the two. This suggests that while these habitats at first appear strikingly different, they require similar adaptations on the scale of the microbes. Several additional features, which are absent from their hydrothermal vent relatives, are present in S. denitrificans, and these additional features may be particularly valuable in the sediment habitat. Their formate dehydrogenase complex would enable S. denitrificans to utilize a major by-product of fermentation that would cooccur with it should sediment organic carbon loads be high. The presence of the additional oxidative stress protein (superoxide dismutase) may enable S. denitrificans to cope with diurnal shifts in sediment oxygen concentration, and several additional resistance-nodulation-cell division family efflux pumps relative to what is seen for hydrothermal vent epsilonproteobacteria suggest that survival in marine sediments requires a degree of versatility and defense against environmental insult beyond what is necessary at moderate temperatures at hydrothermal vents.

ACKNOWLEDGMENTS

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory, Univer-
sity of California, under contract W-7405-ENG-48. Genome closure was funded in part by a USF Innovative Teaching Grant (K.M.S.). S.M.S. received partial support through a fellowship from the Hanse Wissenschaftskolleg in Delmenhorst, Germany (http://www.h-w-k.de), and NSF grant OCE-0452333. K.M.S. is grateful for support from NSF grant MCB-0643713. M.H. was supported by a WHOI postdoctoral scholarship. M.G.K. was supported in part by incentive funds provided by the UO-EVPR office, the KY Science and Engineering Foundation (KSEF-787-RDE-007), and the National Science Foundation (EP-0412129).

We thank Marga Bauer and Frank-Oliver Glöckner for helpful advice and for sharing their annotation guidelines at an early stage of this project, as well as three anonymous reviewers for their insightful suggestions.

REFERENCES