UCRL-JRNL-215002

The Genome Sequence of the Obligately Chemolithoautotrophic, Facultatively Anaerobic Bacterium *Thiobacillus denitrificans*

H. R. Beller, P. S. G. Chain, T. E. Letain, A. Chakicherla, F. W. Larimer, P. M. Richardson, M. Coleman, A. P. Wood, and D. P. Kelly

Published February 2006

Published in the *Journal of Bacteriology*

This work was performed under the auspices of the U.S. Department of Energy by University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.

UCRL-JRNL-215002

PREPRINT

To be published in the *Journal of Bacteriology*

The genome sequence of the obligately chemolithoautotrophic, facultatively anaerobic bacterium *Thiobacillus denitrificans*

Harry R. Beller,[1]* P. S. G. Chain,[1,3] T. E. Letain,[1] A. Chakicherla,[1] F. W. Larimer,[2,3] P. M. Richardson,[3] M. Coleman,[1] A. P. Wood,[4] and D. P. Kelly[5]

[1]Lawrence Livermore National Laboratory, Livermore, CA 94551

[2]Oak Ridge National Laboratory, Oak Ridge, TN 37831

[3]U.S. DOE Joint Genome Institute Production Genomics Facility, Walnut Creek, CA 94598

[4]Department of Microbiology, King's College London, London, SE1 9RT, UK

[5]Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

Running title: Genome sequence of *Thiobacillus denitrificans*

Corresponding author:
Lawrence Livermore National Laboratory
P.O. Box 808, L-542
Livermore, CA 94551-0808
E-mail beller2@llnl.gov
Phone (925) 422-0081
Fax (925) 423-7998

Abstract

The complete genome sequence of *Thiobacillus denitrificans* ATCC 25259 is the first to become available for an obligately chemolithoautotrophic, sulfur-compound-oxidizing, β-proteobacterium. Analysis of the 2,909,809-base pair genome will facilitate our molecular and biochemical understanding of the unusual metabolic repertoire of this bacterium, including its ability to couple denitrification to sulfur-compound oxidation, to catalyze anaerobic, nitrate-dependent oxidation of Fe(II) and U(IV), and to oxidize mineral electron donors. Notable genomic features include (1) one to two percent of the genes encoding *c*-type cytochromes, which is greater than for almost all bacterial and archaeal species sequenced to date, (2) genes encoding two [NiFe]hydrogenases, which is particularly significant because no information on hydrogenases has previously been reported for *T. denitrificans* and hydrogen oxidation appears to be critical for anaerobic U(IV) oxidation by this species, (3) a diverse complement of more than 50 genes associated with sulfur-compound oxidation (including *sox* genes, *dsr* genes, and genes associated with the AMP-dependent oxidation of sulfite to sulfate), some of which occur in multiple (up to eight) copies, (4) a relatively large number of genes associated with inorganic ion transport and heavy metal resistance, and (5) a paucity of genes encoding organic-compound transporters, commensurate with obligate chemolithoautotrophy. Ultimately, the genome sequence of *T. denitrificans* will enable elucidation of the mechanisms of aerobic and anaerobic sulfur-compound oxidation by β-proteobacteria, and will help reveal the molecular basis of this organism's role in major biogeochemical cycles (i.e., sulfur, nitrogen, and carbon) and groundwater restoration.

INTRODUCTION

Thiobacillus denitrificans, first isolated by Beijerinck over a century ago (4), was one of the first non-filamentous bacteria ever described to be capable of growth on inorganic sulfur compounds as sole energy sources (47, 49). Characterized by its ability to conserve energy from the oxidation of inorganic sulfur compounds under either aerobic or denitrifying conditions, *T. denitrificans* is the best studied of the very few obligate chemolithoautotrophic species known to couple denitrification to sulfur-compound oxidation (*Thiomicrospira denitrificans* and *Thioalkalivibrio thiocyanodenitrificans* also have this ability; 76, 85). Despite many years of work on the biochemistry of inorganic sulfur compound oxidation by *Thiobacillus thioparus* and *T. denitrificans*, the mechanisms of oxidation and how they are coupled to energy conservation are still not well understood in these β-proteobacteria, relative to the advances made with facultatively chemolithotrophic α-proteobacterial genera, such as *Paracoccus* and *Starkeya* (28, 39, 45, 50). The availability of the complete genome sequence should enable elucidation of the sulfur-oxidation pathway(s) and lead to specifically focused biochemical investigations to resolve these knowledge gaps.

Recent studies have revealed that, in addition to sulfur-compound oxidation, *T. denitrificans* has broader oxidative capabilities that may not contribute to energy conservation, including anaerobic, nitrate-dependent oxidation of certain metals, such as iron (78). The metabolic repertoire of this widely distributed bacterium can influence the carbon, nitrogen, sulfur, and iron cycles in many soil, aquifer, and sediment environments and is particularly relevant to *in situ* bioremediation of contaminated groundwater. Environmentally relevant capabilities of *T. denitrificans* include intrinsic biodegradation of nitrate, one of the most problematic groundwater contaminants worldwide, by anaerobic, nitrate-dependent oxidation of

minerals such as FeS and pyrite (FeS_2) (e.g., 6, 56, 78). *T. denitrificans* is the first and only autotrophic bacterium reported to carry out anaerobic (nitrate-dependent) oxidation of U(IV) oxide minerals (5), which could partially counteract efforts to remediate uranium-contaminated aquifers by *in situ* reductive immobilization [i.e., microbially mediated conversion of relatively soluble U(VI) species to poorly soluble U(IV) minerals]. The intriguing mechanism by which this species can oxidize mineral electron donors that cannot be taken into the cell is currently unknown but its elucidation will be facilitated by availability of the genome.

In this article, we present the complete genome sequence of *T. denitrificans* strain ATCC 25259, the first obligately chemolithoautotrophic, sulfur-oxidizing, β-proteobacterium to be sequenced. We describe some general features of the *T. denitrificans* genome, including recent gene acquisition, as well as genetic components involved in sulfur-compound oxidation, hydrogen metabolism, aerobic respiration, denitrification, autotrophy, central carbon metabolism, and heavy metal resistance.

MATERIALS AND METHODS

Organism source. *T. denitrificans* strain ATCC 25259 was obtained from the American Type Culture Collection (ATCC). This strain was originally isolated by B. F. Taylor in the 1960s (83, 84) and was deposited with the ATCC in 1969, where it was freeze-dried for storage and distribution. For this study, unless noted otherwise, *T. denitrificans* was grown with thiosulfate under denitrifying conditions, as described elsewhere (5).

Sequencing, coding sequence prediction, and annotation. Genomic DNA was isolated from *T. denitrificans* and the complete genome was sequenced as described previously (15). Briefly, small-insert (2-3 kb), medium-insert (6.5-8.5 kb), and large-insert (35-45 kb) libraries were generated by random mechanical shearing of genomic DNA. In the initial random

sequencing phase, approximately nine-fold sequence coverage was achieved. The sequences from all libraries were assembled together and viewed using the Phred/Phrap/Consed suite (P. Green, University of Washington; 22, 23, 31). Physical gaps were closed by PCR and sequencing. Open reading frames likely to encode proteins (CDS, or coding sequences) were identified and annotated by automated and manual curation as previously described (15).

Comparative genomics. The Integrated Microbial Genomes (IMG) system of the Joint Genome Institute (http://img.jgi.doe.gov/) was used for identification of orthologs and to identify CDS unique to *T. denitrificans* based on BLASTP; the cutoff values applied were $E < 10^{-5}$ and 30% identity, and $E < 10^{-2}$ and 20% identity, respectively.

Generation of phylogenetic trees and other analyses. Phylogenetic trees were generated by identifying potential homologs of translated *T. denitrificans* CDS using BLASTP (1) searches against the non-redundant (nr) GenBank database from the National Center for Biotechnology Information. Typically, trees included the top 50 BLASTP matches. However, sequences were excluded if their BLASTP E values fell below a cutoff of 10^{-5}. For the phylogenetic trees presented in this article, the lowest ranking sequences shared 24 to 54% identity and 43 to 71% similarity with the query sequences. Sequences were aligned and alignments refined using ClustalX (38) version 1.8 and manual adjustments. Phylogenetic trees were generated by using the *protdist* program and the *neighbor* program of the PHYLIP package (24, 25) to calculate distances (using the Jones-Taylor-Thorton matrix) and for clustering (Neighbor-Joining method), respectively. Membrane-spanning domains of proteins were identified using TmHMM (52, 75). The SignalP program (7) was used to identify putative signal peptides.

Reverse transcription, quantitative PCR analysis. For 18 selected genes, mRNA levels under exposure conditions of interest were determined by reverse transcription (RT), quantitative PCR analysis. The target genes included Tbd0210, 0561, 0562, 0822, 0823, 0872, 0873, 0874, 1365, 1408, 1926, 2282, 2283, 2326, 2327, 2480, 2488, 2658. Cultures of *T. denitrificans* were grown anaerobically with thiosulfate and nitrate and were then anaerobically harvested by centrifugation and washed using techniques and conditions described elsewhere (5). Washed cells (~6.5 mg protein) were resuspended in stoppered serum bottles under strictly anaerobic conditions in 5 or 10 mL of bicarbonate-buffered medium. Cells were resuspended either with thiosulfate (20 mM) and nitrate (20 mM) or $FeCO_3$ (3.5 mmol/L) and nitrate (3.5 mM). Chemical analyses (ion chromatography; spectrophotometric determination of ferrozine-iron complexes) of the resuspended cultures were performed to confirm that nitrate reduction and either thiosulfate or Fe(II) oxidation were occurring when the cultures were harvested for RNA. RNA extraction was carried out with a MasterPure Complete DNA and RNA Purification Kit (EpiCentre) using a modified protocol. Total RNA was reverse transcribed and amplified using a QuantiTect SYBR Green RT-PCR kit (Qiagen) with gene-specific primers. Each gene-specific PCR was performed in triplicate using 25-μL reactions containing ~20 ng of template on a Prism 7000 cycler (ABI). Calibration curves were performed with genomic DNA serially diluted over a range of four to five orders of magnitude. The PCR conditions were optimized to be performed as follows for all transcripts: 30-35 cycles at 50°C for 30 minutes; 95°C for 15 minutes; 94°C for 15 seconds; 58°C for 30 seconds; 72°C for 30 seconds.

Nucleotide sequence accession number. The annotated genome sequence has been deposited in the GenBank/EMBL sequence database under accession no. CP000116.

RESULTS AND DISCUSSION

General genome features. The genome of *T. denitrificans* strain ATCC 25259 consists of a single circular chromosome of 2,909,809 bp in length with an average G+C content of 66.1% (Fig. 1 and Table 1). GC skew analysis does not reveal the origin of replication. Nucleotide position 1 of the chromosome is assigned to the predicted origin of replication, flanked on one side by the *dnaA* (Tbd0001), *dnaN* (Tbd0002), and *gyrB* (Tbd0003) genes and on the other by *rpmH* (Tbd2827) and *rnpA* (Tbd2826). Two copies of the 16S ribosomal RNA operon are located in regions of the genome separated by more than 1380 kb. Few other repeated sequences were discovered in the genome, with the exception of a duplicated IS4-like insertion sequence. Thus, the numbers of repeated elements, IS elements, and transposons are low compared with those typically found in bacterial genomes sequenced to date.

A total of 2827 protein-encoding genes were identified, with an average length of 952 bp, accounting for 92.5% of the sequence. Among the predicted genes, 2183 (77.2%) have been assigned a putative function, with the remainder designated as encoding a protein with unknown function, a conserved hypothetical protein, or a hypothetical protein. Of these, 89 predicted genes have no match using BLAST vs. the non-redundant (nr) database with an E value cutoff of 10^{-6} or better. When distributed into biological role categories based on Clusters of Orthologous Groups of proteins (COGs) (82), the largest number of predicted proteins fell into the categories of Energy Production (6.6%), Cell Envelope Biogenesis (6.0%), and Inorganic Ion Transport (5.7%).

When searched against the KEGG database of complete genomes, more than half (1624) of the predicted proteins revealed top BLAST hits to one of the twelve β-proteobacteria available in this database, including *Azoarcus* sp. EbN1 (626 hits), the obligate chemolithoautotrophic

bacterium *Nitrosomonas europaea* (346 hits), *Chromobacterium violaceum* (236 hits), *Ralstonia solanacearum* GMI1000 (189 hits), *Burkholderia pseudomallei* (118 hits), and *Bordetella bronchiseptica* (61 hits). Aside from β-proteobacteria, the organisms most frequently associated with the top BLAST hits were *Methylococcus capsulatus* (107 hits), *Pseudomonas aeruginosa* (75 hits), an environmentally versatile γ-proteobacterium, *Pseudomonas putida* (42 hits), and *Geobacter sulfurreducens* (42 hits), a δ-proteobacterium best known for its versatility in dissimilatory metal reduction.

Recent gene acquisition. There is ample evidence of horizontal transfer in the *T. denitrificans* genome, inferred from local base composition to have been imported from an evolutionarily distant source. At least 13 regions, up to 25 kb in size, have been identified [based on anomalies in observed G+C content and trinucleotide signature (40)] as likely recent integration events into the *T. denitrificans* genome that have not had time to drift toward the genome average (Table 2). Almost all of these putative regions of horizontal gene transfer carry phage integrases or other phage remnants and a few of these regions are also flanked by tRNA genes, which are used as integration sites for many bacteriophages. Most of these regions are found to harbor many hypothetical or conserved hypothetical genes, several carry restriction modification systems, which are known to be associated with mobile elements and indeed act as selfish mobile genetic elements themselves (51), whereas another region carries a cluster of genes encoding part of a type IV pilus. Although these likely recent insertions in the *T. denitrificans* chromosome are consistent with the concept of a fluid genome, it remains to be shown what role these regions may play in the metabolic or defense repertoire of *T. denitrificans*.

Taxonomy of *T. denitrificans* strain ATCC 25259. Following the reclassification of numerous α-, β-, and γ-proteobacterial species previously classified as "*Thiobacillus*", only three

β-proteobacteria are now securely placed in that genus: *T. thioparus, T. denitrificans*, and *T. aquaesulis* (47, 49). Of these, the last is a moderate thermophile and only *T. denitrificans* is capable of strictly chemolithotrophic anaerobic growth with denitrification using inorganic sulfur-compound oxidation as the sole source of energy (46, 49). Apart from this feature, *T. denitrificans* and *T. thioparus* are physiologically very similar mesophilic obligate chemolithoautotrophs. The G+C content of different strains range between 62-67% for *T. thioparus* and 63-68% for *T. denitrificans* (46, 79, 88). DNA hybridization distinguished the two species, which show only 22-29% cross-hybridization (46). Comparison of 16S rRNA gene sequences in the GenBank nr database shows that identities between different strains of *T. thioparus* range between 95.2-99.6%, and that the type strains of *T. thioparus* (ATCC 8158) and *T. denitrificans* (NCIMB 9548) show 96.3% identity to each other (D.P. Kelly, unpublished data). Consequently, the ability to denitrify is a key physiological criterion in distinguishing between the species, given the similarity of their 16S rRNA gene sequences. *T. denitrificans* strain ATCC 25259 was originally isolated from Texas soil (84; ATCC catalog) and has been shown to have physiological properties characteristic of this species. *T. denitrificans* ATCC 25259 shows 97.6% identity to the type strain *T. denitrificans* NCIMB 9548 (with respect to 16S rRNA genes).

Sulfur-compound oxidation. A number of enzymes involved in inorganic sulfur-compound oxidation and energy conservation have previously been studied in *T. denitrificans* and the closely related β-proteobacterium, *T. thioparus*, some of which have been purified and characterized biochemically. The *T. denitrificans* enzymes include APS (adenylylsulfate) reductase, an AMP-independent sulfite oxidase, a siroheme sulfite reductase, and APS:phosphate adenylyltransferase (or APAT) (2, 12, 13, 29, 69-71). Biochemical studies, as well as the high

growth yields and the entry into the electron transport chain of electrons from sulfur-compound oxidation at the level of quinone/cytochrome *b*, indicate highly efficient energy-conserving mechanisms of sulfur-compound oxidation in *T. denitrificans* (42-45). The mechanisms of inorganic-sulfur oxidation by *T. denitrificans*, which oxidizes polythionates as well as thiosulfate, sulfide and, for some strains, thiocyanate, appear more similar to those of γ-proteobacteria such as *Halothiobacillus* than to the well-defined thiosulfate-oxidizing, multienzyme system of α-proteobacterial chemolithotrophs (45, 50, 68).

As would be expected from previous biochemical work with *T. denitrificans*, its genome contains a diverse complement of genes encoding enzymes that catalyze inorganic sulfur-compound oxidation and energy conservation (by both substrate-level and electron transport-linked phosphorylation). The importance of sulfur-compound oxidation to *T. denitrificans* is underscored by the occurrence of multiple oxidation pathways for certain sulfur compounds and multiple copies of a number of genes associated with sulfur-compound oxidation (see overview in Fig. 2). The unusual ability of this bacterium to oxidize inorganic sulfur compounds under both aerobic and denitrifying conditions raises the question of whether different sulfur-oxidizing enzymes are involved under aerobic vs. anaerobic conditions. Although such questions cannot be resolved by genome analysis alone, they can be addressed by transcriptional studies using whole-genome oligonucleotide microarrays, which are now enabled by the availability of the *T. denitrificans* genome. Included among the genes that are likely to be critical to sulfur-compound oxidation by *T. denitrificans* are *sox* (sulfur-oxidation) genes, *dsr* (dissimilatory sulfite reductase) genes, and genes associated with the AMP-dependent oxidation of sulfite to sulfate (Fig. 2). These genes serve as the focus of this section.

Little molecular genetic work on the sulfur-oxidation (*sox*) systems of *T. denitrificans* has been conducted to date. The model for genes encoding sulfur-oxidation enzymes has been derived primarily from three α-proteobacteria: *Paracoccus pantotrophus, Starkeya novella*, and *Pseudaminobacter salicylatoxidans*. In *T. denitrificans*, genes showing various levels of sequence identity to *sox* genes of these α-proteobacteria have been detected, but gene clusters of the length found in facultatively chemolithotrophic, aerobic, thiosulfate-oxidizing bacteria do not occur. Thus, extensive *sox* clusters have been observed in *P. pantotrophus, S. novella*, and *Psb. salicylatoxidans*: *soxRSVWXYZABCDEFGH*, *soxFDCBZYAXWV* and *soxGTRSVWXYZABCD*, respectively (GenBank X79242, AF139113, AJ404005; 27, 28, 91). In contrast, the largest cluster in *T. denitrificans* consists of *soxXYZAB* (Tbd0567-Tbd0563), using the *P. pantotrophus* naming scheme. Encoded polypeptide sequence identities compared to the *soxX,Y,Z,A,B* of *P. pantotrophus, Chlorobium tepidum, Psb. salicylatoxidans*, and *S. novella* were in the range of 28-55%. This indicated that while the genes coding for these Sox functions had been putatively identified, they differed significantly from those of the reference organisms. Interestingly, the translated *soxXYZA* genes showed higher identities to those of the green sulfur bacterium *C. tepidum* (42-55%) than to those of *Paracoccus, Starkeya* and *Pseudaminobacter* (28-38%). Additional copies of *soxXA* were identified outside of the *soxXYZAB* cluster in *T. denitrificans* (Tbd0917-0918). A noteworthy difference between SoxA encoded in the *T. denitrificans* genome and those of *P. pantotrophus* and *Psb. salicylatoxidans* is that the latter are diheme cytochromes (28) whereas SoxA copies in *T. denitrificans* (Tbd0564, and putatively Tbd0918) are monoheme cytochromes.

For *soxB*, the *T. denitrificans* sequence can be compared to another β-proteobacterium as well as to α-proteobacteria, because the sequence from *T. thioparus* is available (59; GenBank

AJ294326; partial sequence – 344 translated amino acids). In fact, of the *sox* genes, only *soxB* has thus far been the subject of intercomparison across the α-, β- and γ-proteobacterial groups (59); this comparison revealed a distant phylogenetic relationship of the *soxB* sequence of *T. thioparus* to those of α- and γ-proteobacteria. Consistent with phylogenetic relationships based on 16S rRNA, the predicted SoxB sequence of *T. denitrificans* (Tbd0563) is much more similar to that of *T. thioparus* (88% identity) than to those of *P. pantotrophus, S. novella*, and *Psb. salicylatoxidans* (48-50% identity). The encoded SoxB sequences for *T. thioparus* and *P. pantotrophus* (GenBank CAC82470 and CAA55824) showed 50% identity to each other.

Other *sox* genes found in the contiguous cluster of *P. pantotrophus* were remote from each other in the *T. denitrificans* genome. These included tentatively identified copies of *soxH* (Tbd1041, Tbd1103), *soxE* (Tbd2027, Tbd2034), *soxF* (Tbd2035), and *soxW* (Tbd2117). Tbd2349 corresponded to the *soxC* (sulfite dehydrogenase) of *Pseudaminobacter* and *Paracoccus*, and the *sorA* of *Starkeya*; thus, the encoded protein may be a sulfite dehydrogenase, catalyzing AMP-independent oxidation of sulfite to sulfate. BLAST probing of the genome with the nucleotide and encoded amino acid sequences of the *soxD* of *P. pantotrophus* and *S. novella* produced no hits, so a gene corresponding to the α-proteobacterial *soxD* appeared to be absent. As the proteins encoded by *soxCD* in *Paracoccus* are believed to catalyze the oxidation of sulfane-sulfur to the oxidation level of sulfite (28), an alternative system is likely present in *T. denitrificans.*

Coding functions have been ascribed to many of the α-proteobacterial *sox* genes discussed above (27, 28, 63-65, 67, 68). *soxX* and *soxA* encode SoxXA, a heterodimeric *c*-type cytochrome. *soxY* and *soxZ* encode SoxYZ, the "thiosulfate-binding" Enzyme A of *P. pantotrophus*. *soxC* and *soxD* encode the molybdoprotein SoxC and diheme *c*-type cytochrome

SoxD of an $\alpha_2\beta_2$-heterodimeric "sulfur dehydrogenase"; SoxCD has also been shown to function as a sulfite dehydrogenase. *soxB* encodes SoxB, a sulfur-thiol esterase, identified in *P. pantotrophus* as Enzyme B, a protein with a dinuclear manganese center that catalyzes thiosulfate cleavage and sulfate production. *soxE* encodes a *c*-type cytochrome. *soxF* encodes a sulfide dehydrogenase/flavocytochrome-*c* oxidoreductase protein. *soxRS* are reported to have a regulatory function. *soxGH* are currently of uncertain function. Finally, the SoxFGH proteins (which are all periplasmic) were reportedly not required for lithotrophic growth of *P. pantotrophus* on thiosulfate, although they were induced by thiosulfate. The proteins SoxXA, SoxYZ, SoxB, and SoxCD can be reconstituted into a system catalyzing thiosulfate-, sulfite-, sulfur-, and hydrogen sulfide-dependent cytochrome *c* reduction in *P. pantotrophus* and *P. versutus*, although this multienzyme system may not operate in the facultative chemolithotroph *S. novella*. The *soxB* gene has also been identified in *T. thioparus*, some γ-proteobacteria (including *Halothiobacillus* and *Thiomicrospira*), and some phototrophic sulfur bacteria (59).

In *T. denitrificans*, central roles for the putative SoxXA, SoxB, and SoxYZ gene products are suggested by the clustering of the genes encoding them, but the low polypeptide sequence identity of these to the corresponding sequences in *P. pantotrophus* could mean that their biochemical functions might differ considerably from those of *P. pantotrophus*. The thiosulfate-oxidizing multienzyme system of *P. pantotrophus* (and of other α-proteobacteria) is located in the periplasm, but there is considerable evidence from *T. denitrificans* (and other obligately chemolithotrophic sulfur oxidizers) that at least some reactions of thiosulfate, sulfite, and sulfide oxidation require membrane-associated processes (43). Deduction of the functions of the putative *T. denitrificans sox* genes solely by reference to the roles of the *sox* complexes in *Paracoccus* species must clearly be done with caution, because even if they were acquired primordially by

lateral gene transfer (59), they could encode significantly modified enzyme functions in extant α- and β-proteobacteria.

In a few bacteria that oxidize inorganic sulfur compounds, namely *T. denitrificans,* *Allochromatium vinosum*, and *C. tepidum*, a siroheme-containing sulfite reductase has been proposed to catalyze the oxidation of certain inorganic sulfur species (e.g., hydrogen sulfide or sulfane-sulfur derived from thiosulfate) to sulfite (20, 71, 86). Thus, dissimilatory sulfite reductase, which is encoded by *dsr* genes and named for its catalytic role in sulfate-reducing bacteria and archaea, is apparently used in the reverse direction for dissimilatory oxidation of sulfur compounds. Siroheme-containing sulfite reductase (with an $\alpha_2\beta_2$ structure encoded by *dsrAB*) was previously purified from *T. denitrificans* strain DSM 807 (86). A gene cluster *dsrABEFHCMKLJOPNR* (Tbd2485-2472) occurs in *T. denitrificans* ATCC 25259 that is very similar in terms of gene sequence and organization to a *dsr* cluster in *A. vinosum* that was studied by Dahl et al. (17). A notable difference in gene organization is that *dsrS* in *T. denitrificans* (Tbd2558) is not part of the *dsr* cluster, as it is in *A. vinosum*, and that a *cysG* (siroheme synthase)-like gene is located adjacent to *dsrR* in *T. denitrificans* (Tbd2471). For the translations of most of these *dsr* genes, the degree of identity with the corresponding *A. vinosum* sequences is >55%. This is the only major cluster of *dsr* genes in the finished *T. denitrificans* sequence, which contrasts with the claim by Dahl et al. (17) that there are two *dsr* gene clusters (based on their examination of shotgun clone sequences in GenBank).

A noteworthy finding with regard to *dsr* genes in *T. denitrificans* was that two genes, *dsrC* and *dsrA*, are replicated multiple times in the genome, typically with no more than one or two adjacent genes that are putatively associated with sulfur-compound oxidation. Eight putative copies of *dsrC* were identified (Fig. 3): Tbd2480 (located in the large *dsr* gene cluster),

Tbd2488 (located next to Tbd2489, which encodes a rhodanese-related sulfurtransferase), Tbd2658, Tbd2326 and 2327 (which are adjacent to each other), Tbd1365 (located near a *dsrA* copy, Tbd1369), Tbd1408 (located next to Tbd1407, which encodes sulfide:quinone oxidoreductase), and Tbd1926. The degree of sequence identity among the corresponding predicted DsrC copies ranges from 26 to 88%. DsrC is a soluble, cytoplasmic protein whose function is not currently known (62). A cysteine residue at the C-terminus of DsrC that is highly conserved in a range of bacteria including *A. vinosum* and various sulfate-reducing bacteria (62) is present in only two of the eight copies encoded in the *T. denitrificans* genome, including the copy located in the *dsr* cluster (Tbd2480) and Tbd2658. In fact, overall, the translated DsrC from Tbd2480 was more similar to DsrC in *A. vinosum* than to the other seven copies in *T. denitrificans* (Fig. 3). Inasmuch as Tbd2480 is the most likely *dsrC* copy in *T. denitrifican*s to code for functional DsrC, it is noteworthy that, like *dsrC* in *A. vinosum* (17, 62) and unlike all other *dsrC* copies in *T. denitrificans*, Tbd2480 appears to be constitutively expressed (based on quantitative, RT, real-time PCR results for *T. denitrificans* carrying out thiosulfate or Fe(II) oxidation under denitrifying conditions; Table 3). As shown in Table 3, Tbd2480 is relatively highly expressed when oxidizing either thiosulfate or Fe(II), whereas none of the other seven putative *dsrC* copies is highly expressed under both conditions.

Three putative copies of *dsrA* were identified in the *T. denitrificans* genome: Tbd2485 (located in the large *dsr* gene cluster), Tbd1309, and Tbd1369 (located near a *dsrC* copy). The degree of sequence identity among the corresponding predicted DsrA copies was high (from 78 to 83%). As *dsrA* codes for the α subunits of the $\alpha_2\beta_2$-structured siroheme sulfite reductase, it is curious that two copies of *dsrA* in the *T. denitrificans* genome are not located near copies of *dsrB*, which codes for the β subunits; indeed, only one *dsrB* copy was identified in the genome

(Tbd2484). Thus, the nature of *dsr* gene duplication in *T. denitrificans* differs from that observed in *C. tepidum*, whose genome includes two copies of the *dsrCABL* cluster (17, 20).

T. denitrificans ATCC 25259 contains genes encoding all the enzymes necessary to catalyze the AMP-dependent oxidation of sulfite to sulfate (Fig. 2). All but one of these enzymes is used in the reverse direction by sulfate-reducing bacteria for the activation of sulfate to APS and the subsequent AMP-yielding reduction of APS to sulfite. APS reductase, an αβ-heterodimeric iron-sulfur flavoenzyme, is encoded by Tbd0872-0873 and catalyzes the AMP-dependent oxidation of sulfite to APS. Another pair of genes, Tbd2282-2283, also putatively encode APS reductase, but quantitative, RT, real-time PCR results suggest that the Tbd0872-0873 genes are much more highly expressed during thiosulfate oxidation (Table 3). ATP sulfurylase, which catalyzes an ATP-yielding substrate level phosphorylation that converts APS to sulfate, is encoded by Tbd0874 and also by Tbd0210 (with the former being more highly expressed; Table 3). APS:phosphate adenylyltransferase (or APAT), which is encoded by Tbd0601 and catalyzes an alternative substrate-level phosphorylation that converts APS to sulfate (yielding ADP rather than ATP), is not reversible (although it was formerly misnamed as ADP sulfurylase) (13).

The *T. denitrificans* genome includes other genes that are likely to play a role in sulfur-compound oxidation, including sulfide-quinone oxidoreductases (Tbd1407, Tbd2225) and rhodanese (thiosulfate-sulfurtransferase; Tbd1650, Tbd2399, Tbd2489). Genes for dimethylsulfoxide (DMSO) reductase (Tbd0570-0572) and tetrathionate reductase (Tbd1739-1741) are also present in the *T. denitrificans* genome, but these may have a role as anaerobic electron acceptors rather than in sulfur-compound oxidation. No genes for the α- and β-subunits of DMSO dehydrogenase were detected. The type strain of *T. denitrificans* (NCIMB 9548) and

some strains of *T. thioparus* can oxidize and grow on thiocyanate as a sole electron donor (47), and three genes of *T. thioparus* encode the thiocyanate hydrolase enzyme that initiates thiocyanate degradation (GenBank AB007989, *scnBAC*). In contrast, *T. denitrificans* strain ATCC 25259 does not oxidize thiocyanate, and its genome lacks the genes encoding thiocyanate hydrolase.

Hydrogen metabolism. Analysis of the genome of *T. denitrificans* ATCC 25259 has revealed the presence of genes encoding two [NiFe]hydrogenases. Hydrogenases are metalloenzymes that catalyze the reversible oxidation of H_2 to protons and are vital components of the energy metabolism of many microbes. Notably, hydrogenases have not previously been reported in *T. denitrificans*, and the sequenced strain does not appear to be able to grow on hydrogen as a sole electron donor under denitrifying conditions (H. Beller, unpublished data); however, hydrogen oxidation appears to be required for nitrate-dependent U(IV) oxidation by *T. denitrificans* (5).

One of the hydrogenases encoded in the *T. denitrificans* genome is putatively a cytoplasmic, heterotetrameric, Group 3b [NiFe]hydrogenase [following the classification system described by Vignais et al. (87)]. The four-gene cluster (Tbd1260-1263) does not appear to be near any accessory or maturation genes encoding proteins necessary for assembly of the functional holoenzyme. Although Group 3b hydrogenases have primarily been found in hyperthermophilic archaea (87), BLASTP analysis revealed that a group of similar predicted proteins (43 to 53% identity for the four subunits) occur in *Azotobacter vinelandii*, a mesophilic, δ-proteobacterium; gene organization in *T. denitrificans* and *A. vinelandii* was also similar. Group 3b hydrogenases in the hyperthermophile *Pyrococcus furiosus* are among the better characterized and are thought to play a role in disposing of excess reductant generated during

fermentation (whereby NADPH can serve as the physiological electron donor for H_2 evolution)(87). The role of a Group 3b hydrogenase in *T. denitrificans*, if indeed this hydrogenase is expressed in functional form, is not currently known.

The other hydrogenase encoded in the *T. denitrificans* genome is putatively a periplasmic, Group 1 [NiFe]hydrogenase [following the classification system described by Vignais et al. (87)]. Although the sequences of the small (HynS) and large (HynL) subunits of this hydrogenase are similar to those in many bacteria and archaea, they occur in an unusual gene cluster (*hynS-isp1-isp2-hynL*; Tbd1378-1375) that has only been observed in four other microbes to date, none of which is a mesophilic, chemolithoautotrophic bacterium like *T. denitrificans*: the phototrophic sulfur bacteria *Thiocapsa roseopersicina* (66) and *A. vinosum* (16), the hyperthermophilic bacterium *Aquifex aeolicus* (GenBank NP 213658.1-213655.1), and the archaeon *Acidianus ambivalens* (53). Typically, *hynS* and *hynL* are adjacent to one another, but they are separated by two intervening genes in these species. Not only is the organization of the *hynS-isp1-isp2-hynL* gene cluster identical in *T. denitrificans* and these other four species, the sequence similarity of the four predicted proteins is also relatively high among these species (Fig. 4). The degree of sequence identity for HynS, Isp1, Isp2, and HynL in *T. denitrificans* compared to *T. roseopersicina*, *A. aeolicus*, and *A. vinosum* ranges from 26 to 68%. A phylogenetic tree of Isp1 in *T. denitrificans* ATCC 25259 and the small complement of related proteins from the GenBank nr database (BLASTP E values < 10^{-5}) reveals similarity not only to Isp1 copies of other species but also to NarI (the γ subunit of cytoplasmic nitrate reductase) and Hmc5 (part of the high-molecular-weight, transmembrane, electron transport protein complex found in *Desulfovibrio vulgaris*)(Fig. 4B). Alignment of the predicted amino acid sequence of Isp1 from *T. denitrificans* with other sequences represented in Fig. 4B shows that four histidine

residues (located in two of the five predicted transmembrane helices in Isp1, NarI, and Hmc5) are highly conserved. Similar observations have been made for other Isp1 sequences (16, 53, 66) and Berks et al. (8) elucidated how the conserved histidines (and the two *b*-hemes that they putatively bind, one in each half of the membrane bilayer) play a role in mediating transport of electrons across the cytoplasmic membrane as part of an electron-carrying arm of a redox loop in the integral membrane proteins NarI or HyaC (a *b*-type cytochrome associated with periplasmic [NiFe]hydrogenases). Based on these and other observations, it is likely that Isp1 serves two functions: (1) to anchor HynSL on the periplasmic side of the cytoplasmic membrane, where H_2 can be converted to $2H^+$ and 2 e^- by HynSL, and (2) to mediate transmembrane electron transfer from HynSL to the quinone pool of electron transport chains and thereby participate in a chemiosmotic mechanism of energy conservation. Notably, in serving these functions, Isp1 may be substituting for the *b*-type cytochrome HupC/HoxZ/HupZ/HyaC that is typically found in Group 1 [NiFe]hydrogenases but which is apparently absent in the *T. denitrificans* genome. A phylogenetic tree of Isp2 in *T. denitrificans* (Fig. 4C) reveals a similarity not only to Isp2 sequences of other species but also to various iron-sulfur-containing proteins such as heterodisulfide reductases, DsrK (from other sulfur-compound oxidizers *A. vinosum* and *C. tepidum*), and Hmc6 from *D. vulgaris*; such similarities have been noted previously for other Isp2 copies (16, 66).

The two adjacent gene clusters (Tbd1380-1374 and Tbd1381-1386; on opposite DNA strands) that code for the Group 1 [NiFe]hydrogenase of *T. denitrificans* include a number of genes putatively involved in biosynthesis and maturation of the hydrogenase. This clustering of maturation genes along with the *hynS-isp1-isp2-hynL* cluster in *T. denitrificans* distinguishes its gene organization from that of *T. roseopersicina* and *A. aeolicus*, which do not have accessory

genes in the immediate vicinity of the *hynS-isp1-isp2-hynL* cluster. In the Tbd1380-1374 gene cluster, Tbd1374 codes for a homolog of *UreJ* and is probably involved in Ni transport; *hynD* (Tbd1380) codes for a putative maturation protease. Most or all of the genes in the *hypCABDFE* gene cluster (Tbd1381-1386) encode proteins that are putatively involved with insertion of Ni, Fe, CO, and CN in the active site of the hydrogenase (87).

Aerobic respiration. The genome of *T. denitrificans*, a facultative anaerobe, encodes all the necessary machinery for aerobic respiration, including NADH:ubiquinone oxidoreductase (complex I; Tbd1142-1155), succinate dehydrogenase (complex II; Tbd1182-1185), and cytochrome bc_1-type ubiquinol oxidoreductase (complex III; Tbd1831-1833) (9). These components can provide the reducing equivalents needed for terminal respiration with nitrate or oxygen, the latter in conjunction with one of three terminal cytochrome *c* oxidases encoded by two gene clusters on the *T. denitrificans* genome. The first cluster encodes a cytochrome aa_3-type cytochrome *c* oxidase (Tbd0325, 0326, 0328, 0330) and a cbb_3-type cytochrome *c* oxidase (Tbd0338-0341), whereas the second cluster (Tbd0640-0643) encodes a second cbb_3-type cytochrome *c* oxidase. The presence of both aa_3- and cbb_3-type oxidases, in addition to the denitrification machinery, allows *T. denitrificans* to survive under a wide range of redox conditions; presumably, the aa_3 oxidase operates under high oxygen tension, cbb_3 oxidases operate under microaerophilic conditions, and the denitrification complex operates under anaerobic conditions (58, 61).

Denitrification. *T. denitrificans* has all necessary genes encoding the four essential enzymes that catalyze denitrification (reduction of nitrate to nitrogen gas): nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase (60, 94). The membrane-bound, dissimilatory nitrate reductase is encoded in a *narKK*$_2$*GHJI* cluster (Tbd1401-1406),

whereas the NarXL two-component regulatory system is encoded on the reverse DNA strand (Tbd1400-1399). This gene organization is similar to that described for *Pseudomonas aeruginosa* PAO1 and *P. fluorescens* C7R12 (60). A *nir* operon including the CDS for cytochrome cd_1-nitrite reductase (*nirS*; Tbd0077) is present in *T. denitrificans*, and in fact, this protein was purified from a different strain of *T. denitrificans* (strain DSM 807; 36). *T. denitrificans* ATCC 25259 contains two *nor* gene clusters that include the *norCB* structural genes encoding nitric oxide reductase, a membrane-anchored protein complex. Quantitative, RT, real-time PCR results carried out with thiosulfate-oxidizing, denitrifying cells (Table 3) indicated that the *norCB* genes Tbd0562-0561 had >50-fold higher expression levels than *norCB* genes Tbd0822-0823, strongly indicating that the former genes are of greater functional importance. The predicted NorC amino acid sequence for the less-expressed copy (Tbd0822) contains >120 more residues at the C-terminus than the highly expressed copy (Tbd0562) or a similar copy in *Azoarcus* sp. EbN1 (GenBank YP_157125); these additional amino acid residues for Tbd0822 include a second CXXCH heme-binding motif. The enzyme catalyzing the final step of denitrification, nitrous oxide reductase, is associated with the structural gene *nosZ* (Tbd1389), and, like NirS, was purified from *T. denitrificans* strain DSM 807 (36). Organization of *nos* genes in *T. denitrificans* is similar, but not identical, to that described for *Ralstonia metallidurans* CH34 (60). As has been observed for some other β-proteobacteria (60), *nosR* (Tbd1390) is located downstream from *nosZ* in *T. denitrificans*.

***c*-Type cytochromes**. *T. denitrificans* strain ATCC 25259 has a higher number of *c*-type cytochromes than most bacteria with finished genomes, based on analysis of the characteristic CXXCH heme-binding motif throughout the genome. Fifty-six genes, or approximately 2% of the total CDS, contained at least one CXXCH motif (some encoded proteins, such as DnaJ and

ribosomal protein L31, are not truly *c*-type cytochromes; Table 4). In the context of bacteria reported to have relatively high numbers of *c*-type cytochromes, the number in *T. denitrificans* is less than that cited for *Geobacter sulfurreducens* (111, or 3.2% of the total CDS) (54) but more than for *Shewanella oneidensis* (39, or 0.8% of the total CDS)(34) and *Pseudomonas aeruginosa* (35, or 0.6% of the total CDS)(77). Overall, *c*-type cytochromes in *T. denitrificans* range in predicted molecular mass from 9.5 to 138 kDa and in number of heme groups from one to three (with only one triheme *c*-type cytochrome and the vast majority as monoheme proteins)(Table 4). Although the functions of some *c*-type cytochromes in *T. denitrificans* can be confidently predicted, such as NirS and NorC, some are of less certain or unknown function (Table 4). The ability of *T. denitrificans* to catalyze anaerobic, nitrate-dependent oxidation of metals with high reduction potentials, such as Fe(II) or U(IV) (5, 78), may be mediated by *c*-type cytochromes, as few other electron carriers in a bacterial cell would have sufficiently high reduction potentials to accept electrons from compounds such as uraninite, or UO_2 (the UO_2^{2+}/UO_2 couple has an E_0' value of +0.26V; 5).

Autotrophy. The genome of this obligate chemolithoautotroph encodes both Form I and Form II ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzymes for CO_2 fixation (21, 35). The Form I genes (*cbb*L and *cbb*S) occur in an operon with *cbb*Q and *cbb*O genes (*cbb*LSQO; Tbd2624-2621). The Form II gene, *cbb*M, is in a separate operon that also includes *cbb*Q and *cbb*O genes; the operon has the form *cbb*MQO (Tbd2638-2636). Both operons are preceded by divergently transcribed *cbb*R genes encoding LysR-type transcriptional regulators (Tbd2625 and 2639). The *cbb*Q and *cbb*O genes encode proteins involved in the post-translational activation of RuBisCO (33). The two copies of each gene are quite distinct: the two CbbQ proteins have 71% identical residues, whereas the two CbbO proteins only share 34%

identity. The two RuBisCO operons, although separate, fall within a 33-kb region that also includes an operon of ten genes (Tbd2641-2650) encoding the carboxysome shell proteins (14). The carboxysome operon does not begin with *cbb*LS, unlike other known examples (14). These three operons, all located on the reverse strand, are oriented to be transcribed in the same direction. The carboxysome operon includes the gene (Tbd2649) for carbonic anhydrase (CA) epsilon (74). The carboxysome operon is also preceded by a divergently transcribed *cbb*R gene (Tbd2651) encoding a LysR-type transcriptional regulator. In addition to the epsilon-type CA, a eukaryotic-type CA (Tbd2167) is encoded elsewhere in the genome. Since there is no evidence that the carboxysome operon is functional (14, 72), this alternative CA may be the primary source of this activity.

Inasmuch as *T. denitrificans* can grow under both aerobic and denitrifying conditions, and Form I and Form II RuBisCO in this species were shown to have markedly different abilities to discriminate between CO_2 and O_2 (35), it is possible that Form I and II RuBisCO are differentially expressed in *T. denitrificans* based on the concentration of O_2. Molecular oxygen competes with CO_2 for the active site of RuBisCO and thereby decreases its efficiency for carbon fixation. Hence, Form I, which has a higher CO_2/O_2 specificity, should be more highly expressed under aerobic conditions, whereas Form II should be more highly expressed under anaerobic conditions.

Genes for all enzymes to complete the Calvin-Benson-Bassham (CBB) cycle are present. Transketolase, NAD-dependent glyceraldehyde 3-phosphate dehydrogenase (E.C.1.2.1.12), phosphoglycerate kinase, pyuvate kinase, and fructose 1,6-bisphosphate aldolase (Tbd0159-0163) are encoded by an operon, whereas fructose 1,6-/sedoheptulose-1,7- bisphosphatase (Tbd2577), ribose 5-phosphate isomerase (Tbd2364), and phosphoribulokinase (Tbd2447) are

encoded by isolated genes. Genes encoding ribulose 5-phosphate 3-epimerase and phosphoglycolate phosphatase (Tbd2230-2229) are adjacent but may not be co-transcribed.

Central intermediary metabolism. Organic storage materials have not been reported in *T. denitrificans*, but the presence of genes encoding glycogen synthase (Tbd2057), maltooligosyl trehalose synthase (Tbd1174), and various glucan branching enzymes (e.g., an α-1,4-D-glucan branching enzyme; Tbd1173, Tbd2058) and glucano- and glycosyl-transferases suggests that the bacterium synthesizes a polyglucose storage product (cf. *Halothiobacillus neapolitanus*; 10). The gene for the key enzyme of the Embden-Meyerhof-Parnas (EMP) pathway necessary for it to effect gluconeogenesis (fructose 1,6-bisphosphatase) is present in the genome (Tbd2577). The synthesis of such a storage product would provide a rationale for the presence of genes for glucokinase (Tbd2062, Tbd2216), which could thus assist in the endogenous catabolism of stored polyglucose. Several genes encoding enzymes for polysaccharide hydrolysis also appear to be present: β-galactosidase (Tbd2429), several glycosidases (Tbd0727, 0923, 1172) and glycosyl transferases (e.g., Tbd0289, 0293, 0294, 0301, 2139), an α-mannosidase (Tbd2060), and an α-arabinofuranosidase (Tbd1789).

Genes encoding all the enzymes of the EMP pathway necessary for it to function in the forward direction for the conversion of glucose to pyruvate are present in the genome, including the gene for phosphofructokinase (Tbd1502), which is unique to the degradative EMP pathway. Genes for all the enzymes of the oxidative pentose phosphate (OPP) pathway for the oxidation of glucose to carbon dioxide are also present in the genome. Some of the enzymes of the EMP and OPP pathways are also common to the CBB reductive pentose phosphate cycle for carbon dioxide fixation, and ribose 5-phosphate for nucleic acid synthesis can be produced from glucose 6-phosphate by glucose 6-phosphate dehydrogenase, phosphogluconolactonase, and 6-

phosphogluconate dehydrogenase (encoded by Tbd2121-2123). In addition, genes encoding alcohol dehydrogenase (EC 1.1.1.1; Tbd1767) and other short-chain alcohol dehydrogenases (Tbd0924, 1469, 1549, 1699, 1886, 2701, 2756), lactate dehydrogenase (Tbd1998), and phosphoketolase (EC 4.1.2.9) (Tbd0049, 0831) suggest that *T. denitrificans* might be able to produce ethanol and lactate (by homo- or hetero-fermentative metabolism) from endogenous glucose under anoxic conditions. This would parallel the heterolactic fermentation of stored polyglucose carried out anaerobically by *Halothiobacillus neapolitanus* (11). Glucose 6-phosphatase and glycerol phosphatase are absent from the *T. denitrificans* genome, reflecting the lack of need to produce free glucose or glycerol.

Genes for some enzymes of the Entner-Doudoroff (ED) pathway are present, but notably absent is the gene encoding 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase, meaning that *T. denitrificans* cannot express a functional ED pathway. We failed to detect KDPG aldolase by BLAST searching of the genome using database polypeptide sequences from *Zymomonas, Escherichia coli, Gluconobacter*, and *Neisseria*. The gene putatively encoding 6-phosphogluconate (6-PG) dehydratase (Tbd2730), whose product is KDPG, may be a false identification, as a TBLASTN search of the *T. denitrificans* genome with the 6-PG dehydratase protein sequence of *Xanthomonas* (GenBank NP_642389) failed to detect any matching sequences, although direct BLAST2(P) comparison of the polypeptide encoded by Tbd2730 did show low identity (27–29%) to the 6-PG dehydratases of *Xanthomonas* and *Helicobacter* (GenBank NP_223740). The highest identities of Tbd2730 indicated by BLAST analyses were to dihydroxyacid dehydratases, and comparisons by BLASTP of the polypeptide sequences of the dihydroxyacid dehydratase of *Sinorhizobium* (GenBank AL591792) with the 6-PG dehydratase

of *Xanthomonas* also showed 29% identity. Assigning putative function must thus be done with caution.

Genes for all the enzymes of the Krebs tricarboxylic acid cycle were identified in the genome. The E1 and E2 subunits of 2-oxoglutarate dehydrogenase are encoded by Tbd1188 and Tbd1189, whereas the E3 subunit (common also to pyruvate dehydrogenase) is encoded by Tbd0652. Genes encoding the E1, E2 and E3 subunits of pyruvate dehydrogenase are Tbd0652, 0654, and 0655, whereas Tbd1847 putatively encodes all three subunits, with highest identity to the *pdhA*, *pdhB*, and *pdhL* genes of *Ralstonia eutropha*. It was surprising to find genes for the E1 and E2 subunits of 2-oxoglutarate dehydrogenase, as *T. denitrificans* strains (including ATCC 25259) do not express an active 2-oxoglutarate dehydrogenase enzyme when growing autotrophically (57, 83). This inability is shared with other obligate chemolithotrophs and methanotrophs, including *Nitrosomonas europaea* and *Methylococcus capsulatus*, and has been proposed as a contributory factor in the obligate growth modes of these bacteria (15, 41, 48, 73, 89, 90, 92). The E3 component of the pyruvate and 2-oxoglutarate dehydrogenases is also known to be controlled by multiple regulatory mechanisms (93), so it may be that failure of obligate chemolithotrophs to express active 2-oxoglutarate dehydrogenase results from regulation at the E3 gene expression level.

While identifying genes encoding some Krebs cycle enzymes, it was found that a gene (Tbd2119) showed significant identity to both fumarase (fumarate hydratase; EC 4.2.1.2) and aspartate ammonia-lyase (EC 4.3.1.1). BLASTP analysis showed the translated sequence of Tbd2119 to share 55% sequence identity with the aspartate ammonia-lyase of *Geobacter sulfurreducens* (GenBank NP_951538.1), 55% identity with the fumarase sequence of *Pelobacter propionicus* (GenBank ZP_00677090.1), and 41% with the fumarase C of *E. coli*. We

suggest that the role of the Tbd2119 gene product is as a fumarase in the Krebs cycle. This similarity of genes for fumarase and aspartate ammonia-lyase is common across the database sequences for numerous organisms, possibly indicating multifunctional roles for the genes or their encoded proteins.

Genes encoding isocitrate lyase and malate synthase have not been detected using BLASTP searches with the translated polypeptide sequences of the genes from *E. coli*, meaning that the glyoxylate cycle cannot be present and hence exogenous acetate could not be metabolized as a sole source of carbon by that route.

Transport systems for organic nutrients. Permease systems for inorganic ions and numerous ABC transporter components are encoded within the genome, but relatively few specific systems have been detected for the uptake of sugars or organic acids. Those detected include various components constituting a tripartite ATP-independent periplasmic (TRAP)-type C_4-dicarboxylate transporter system (Tbd0466, 0467, 0468, 2151, 2164), and phosphotransferase system components including Enzyme I (Tbd2414), HPr (Tbd2413), two Enzyme IIA subunits specific for mannose/fructose (Tbd2412, Tbd0531), and Hpr(ser) kinase/phosphorylase (Tbd0530). The absence from the *T. denitrificans* genome of a gene encoding Enzyme IIC (the sugar permease component) suggests that the role of this PTS system may be regulatory rather than for sugar transport (30). The genome of *T. denitrificans* also encodes two sodium:solute symporter family proteins that may be involved in acetate uptake (Tbd0088, 0212). A sodium:solute symporter protein, ActP, has been shown to be involved in acetate uptake in *E. coli* (GenBank P32705). A functional transport system for acetate in *T. denitrificans* was indicated by the uptake of ^{14}C-labeled acetate into bacteria growing chemolithotrophically [acetate provided 6-11% of the cell-carbon of strain ATCC 25259 (84)]. As well as being used

for lipid biosynthesis, ^{14}C-acetate was incorporated only into the protein-amino acids glutamate, proline, and arginine, as reported for other obligate chemolithotrophs and methanotrophs that lack 2-oxoglutarate dehydrogenase (19, 41, 73, 92). Incorporation of acetate-carbon into glutamate by *T. denitrificans* was unaffected by exogenous glutamic acid, which was presumably not taken up significantly by the bacteria. The possibility remains to be tested that chemolithoorganotrophic growth by *T. denitrificans* might be possible if the organism were presented with compounds such as acetate, or a suitable C_4-dicarboxylic acid, in the presence of thiosulfate and nitrate as the energy source. This would be akin to the chemolithoorganotrophic growth of *Nitrosomonas europaea* on fructose or pyruvate and ammonia (15, 37).

Inorganic ion transport and heavy metal resistance. A number of genes were identified in *T. denitrificans* that putatively code for transporters that can mediate either the uptake or efflux of a range of inorganic ions. In all, at least 18 complete ABC (ATP Binding Cassette) transporters predominantly for inorganic molecules are present in the genome allowing for the uptake of Fe^{3+}, thiosulfate, nitrate, nitrite, and many other ions (18). Numerous non-ABC type transporters allowing for uptake of other ions, such as various sulfur-containing compounds and bicarbonate, are also present. The genome of *T. denitrificans* also contains a surprisingly large number of metal resistance systems, particularly considering its relatively small genome size (Table 5). In total, the *T. denitrificans* genome encodes as many as 17 possible metal resistance systems [described by Nies (55)] including five heavy metal efflux (HME) systems from the resistance-nodulation-cell division (RND) family of transporters; three cation diffusion facilitators (CDF); three CPx-type ATPases (heavy-metal specific P-type ATPases); and five additional gene clusters encoding possible resistance systems specific for metals such as Ni^{2+} (*nreB*), Pb^{2+} (*pbrT*), Hg^{2+} (3), chromium (as chromate; *chrA*), and Cu^{2+}/Ag^{2+} (26, 32). Although

the *T. denitrificans* genome has fewer systems than the model metal-resistant bacterium *Ralstonia metallidurans* (55), it has more than most other bacteria characterized to date (Table 5). Notably, *T. denitrificans* (or species with >98% 16S rDNA sequence similarity) was found to be prevalent in an inactive uranium mine with relatively high concentrations of uranium, nickel, cobalt, and zinc (80).

Several of the efflux-mediated heavy metal resistance systems, along with various systems involved in metal uptake and storage, are found in large gene clusters on the *T. denitrificans* genome (Fig. 1). The largest of these metal transport clusters, Tbd0704-0726, encodes proteins allowing for high-affinity Fe^{3+} acquisition, Fe^{3+} storage, Pb^{2+} resistance, and heavy metal resistance. Genes involved in high-affinity Fe^{3+} acquisition include homologs of a portion of the *Vibrio parahaemolyticus* polyhydroxycarboxylate-type siderophore biosynthesis, secretion, and uptake gene cluster *pvuApvsABCDE psuA* (Tbd0722-0717, Tbd0715) (81), and *tonBexbBD* (Tbd0713-0711), which allows for active transport across the outer membrane of Fe^{3+}-bound siderophore. Also found are genes encoding the Fe^{3+} storage proteins bacterioferritin and bacterioferritin-associated ferredoxin (Tbd0704-0705), Pb^{2+} resistance (Tbd0723), and CDF family heavy metal resistance (Tbd0726). A second large gene cluster (encompassing genes on both the forward and reverse strands) encodes proteins primarily associated with metal resistance (Tbd1324-1341). This cluster includes a multi-copper oxidase (Tbd1324) (32), a periplasmic Cu^{2+}-binding protein (Tbd1326) (26), two HME-RND systems (Tbd1327-1329, Tbd1333-1335), and a Hg^{2+} resistance system *merRTPA* (Tbd1338-1341) (3).

Future prospects. The availability of the *T. denitrificans* genome has fostered new insights into this bacterium and will effectively focus further investigations into its biochemistry and physiology. Among the new insights reported here are the following genomic

characteristics: an unusually large number of genes encoding *c*-type cytochromes, a relatively large complement of genes associated with inorganic ion transport and heavy metal resistance, and the presence of genes encoding two [NiFe]hydrogenases, which is particularly significant because no physiological, biochemical, or genetic information on hydrogenases has previously been reported for this species. The genome also provides much more information on the genetic basis of sulfur-compound oxidation in chemolithotrophic β-proteobacteria (particularly with respect to *sox* and *dsr* genes). Much more work will be required to understand the genetic and biochemical basis of unusual and enigmatic metabolic capabilities of this bacterium, including the coupling of denitrification with sulfur-compound oxidation, the use of mineral electron donors, and the anaerobic, nitrate-dependent oxidation of metals. Whole-genome transcriptional studies with cDNA microarrays are currently underway to address such questions.

ACKNOWLEDGMENTS

We thank A. Lapidus and C. Detter (DOE Joint Genome Institute), M. Land (Oak Ridge National Laboratory), and M. Shin, T. Legler, F. Bourguet, W. Marrs, and A. Krefft (Lawrence Livermore National Laboratory) for their valuable contributions.

This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.

REFERENCES

1. **Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25**:3389-3402.

2. **Aminuddin, M., and D. J. D. Nicholas.** 1974. An AMP-independent sulphite oxidase from *Thiobacillus denitrificans*: purification and properties. J. Gen. Microbiol. **82**:103-113.

3. **Barkay, T., S. M. Miller, and A. O. Summers.** 2003. Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol. Rev. 27:355-384. FEMS Microbiol. Rev. 27:355-384.

4. **Beijerinck, M. W.** 1904. Phénomènes de réduction produits par les microbes (Conférence avec demonstrations faite – Delft, le 16 avril 1903). Archs. Neerl. Sci. Ser. *2* **9**:131-157.

5. **Beller, H. R.** 2005. Anaerobic, nitrate-dependent oxidation of U(IV) oxide minerals by the chemolithoautotrophic bacterium *Thiobacillus denitrificans*. Appl. Environ. Microbiol. **71**:2170-2174.

6. **Beller, H. R., V. Madrid, G. B. Hudson, W. W. McNab, and T. Carlsen.** 2004. Biogeochemistry and natural attenuation of nitrate in groundwater at an explosives test facility. Appl. Geochem. **19**:1483-1494.

7. **Bendtsen, J. D., H. Nielsen, G. von Heijne, and S. Brunak.** 2004. Improved prediction of signal peptides: SignalP 3.0. J. Mol. Biol. **340**:783-795.

8. **Berks, B. C., M. D. Page, D. J. Richardson, A. Reilly, A. Cavill, F. Outen, and S. J. Ferguson.** 1995. Sequence analysis of subunits of the membrane-bound nitrate reductase from a denitrifying bacterium: the integral membrane subunit provides a prototype for the dihaem electron-carrying arm of a redox loop. Mol. Microbiol. **15**:319-331.

9. **Berlyn, M. K. B., K. B. Low, and K. E. Rudd.** 1996. Linkage map of *Escherichia coli* K12, edition 9, p. 1715-1902. *In* F.C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C.

Lin, et al. (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 2, ASM Press, Washington, DC.

10. **Beudeker, R. F., J. W. M. Kerver, and J. G. Kuenen.** 1981a. Occurrence, structure and function of intracellular polyglucose in the obligate chemolithotroph *Thiobacillus neapolitanus*. Arch. Microbiol. **129**: 221-226.

11. **Beudeker, R. F., W. de Boer, and J. G. Kuenen.** 1981b. Heterolactic fermentation of intracellular polyglucose by the obligate chemolithotroph *Thiobacillus neapolitanus* under anaerobic conditions. FEMS Microbiol. Lett. **12**: 337-342.

12. **Bowen, T. J., F. C. Happold, and B. F. Taylor.** 1966. Studies on the adenosine-5'-phosphosulfate reductase from *Thiobacillus denitrificans*. Biochim. Biophys. Acta **118**:566-576.

13. **Brüser, T., T. Selmer, and C. Dahl.** 2000. "ADP sulfurylase" from *Thiobacillus denitrificans* is an adenylylsulfate:phosphate adenylyltransferase and belongs to a new family of nucleotidyltransferases. J. Biol. Chem. **275**:1691-1698.

14. **Cannon, G. C., S. H. Baker, F. Soyer, D. R. Johnson, C. E. Bradburne, J. L. Mehlman, P. S. Davies, Q. L. Jiang, S. Heinhorst, J. M. Shively.** 2003. Organization of carboxysome genes in the thiobacilli. Curr. Microbiol. **46**:115-119.

15. **Chain, P., J. Lamerdin, F. Larimer, W. Regala, V. Lao, M. Land, L. Hauser, A. Hooper, M. Klotz, J. Norton, L. Sayavedra-Soto, D. Arciero, N. Hommes, M. Whittaker, and D. Arp.** 2003. Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europaea*. J. Bacteriol. **185**:2759-73.

16. **Dahl, C., G. Rakhely, A. S. Pott-Sperling, B. Fodor, M. Takacs, A. Toth, M. Kraeling, K. Gyorfi, A. Kovacs, J. Tusz, and K. L. Kovacs.** 1999. Genes involved in hydrogen and sulfur metabolism in phototrophic sulfur bacteria. FEMS Microbiol. Lett. **180**:317-324.

17. **Dahl, C., S. Engels, A. S. Pott-Sperling, A. Schulte, J. Sander, Y. Lübbe, O. Deuster, and D. C. Brune.** 2005. Novel genes of the *dsr* gene cluster and evidence for close interaction of Dsr proteins during sulfur oxidation in the phototrophic sulfur bacterium *Allochromatium vinosum*. J. Bacteriol. **187**:1392-1404.

18. **Dassa, E., and P. Bouige.** 2001. The ABC of ABCs: a phylogenetic and functional classification of ABC systems in living organisms. Res. Microbiol. **152**: 211-229.

19. **Eccleston M., and D. P. Kelly.** 1973. Assimilation and toxicity of some exogenous C-1 compounds, alcohols, sugars and acetate in the methane-oxidizing bacterium *Methylococcus capsulatus*. J Gen. Microbiol. **75**:211-221.

20. **Eisen, J. A., K. E. Nelson, I. T. Paulsen, J. F. Heidelberg, M. Wu, R. J. Dodson, R. Deboy, M. L. Gwinn, W. C. Nelson, D. H. Haft, E. K. Hickey, J. D. Peterson, A. S. Durkin, J. L. Kolonay, F. Yang, I. Holt, L. A. Umayam, T. Mason, M. Brenner, T. P. Shea, D. Parksey, W. C. Nierman, T. V. Feldblyum, C. L. Hansen, M. B. Craven, D. Radune, J. Vamathevan, H. Khouri, O. White, T. M. Gruber, K. A. Ketchum, J. C. Venter, H. Tettelin, D. A. Bryant, and C. M. Fraser.** 2002. The complete genome sequence of *Chlorobium tepidum* TLS, a photosynthetic, anaerobic, green-sulfur bacterium. Proc. Natl. Acad. Sci. USA **99**:9509-9514.

21. **English, R. S., C. A. Williams, S. C. Lorbach, and J. M. Shively.** 1992. Two forms of ribulose 1,5-bisphosphate carboxylase/oxygenase from *Thiobacillus denitrificans*. FEMS Microbiol. Lett. **73**:111-9.

22. **Ewing, B., and P. Green.** 1998. Basecalling of automated sequencer traces using phred. II. Error probabilities. Genome Research **8**:186-194.

23. **Ewing, B., L. Hillier, M. Wendl, and P. Green.** 1998. Basecalling of automated sequencer traces using phred. I. Accuracy assessment. Genome Research **8**:175-185.

24. **Felsenstein, J.** 1989. PHYLIP - Phylogeny Inference Package (version 3.2). Cladistics **5**:164-166.

25. **Felsenstein, J.** 2002. PHYLIP (Phylogeny Inference Package) version 3.6a3. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

26. **Franke, S., G. Grass, C. Rensing, and D. H. Nies.** 2003. Molecular analysis of the copper-transporting efflux system CusCFBA of *Escherichia coli*. J. Bacteriol. **185**: 3804-3812.

27. **Friedrich, C. G., A. Quentmeier, F. Bardichewsky, D. Rother, R. Kraft, S. Kostka, and H. Prinz.** 2000. Novel genes coding for lithotrophic sulfur oxidation of *Paracoccus pantotrophus* GB17. J Bacteriol. **182**:4677-4687.

28. **Friedrich, C. G., D. Rother, F. Bardichewsky, A. Quentmeier, and J. Fischer.** 2001. Oxidation of reduced inorganic sulfur compounds by bacteria: emergence of a common mechanism? Appl. Environ. Microbiol. **67**:2873-2882.

29. **Fritz, G., T. Büchert, H. Huber, K. O. Stetter, and P. M. H. Kroneck.** 2000. Adenylylsulfate reductases from archaea and bacteria are 1:1 αβ-heterodimeric iron-

sulfur flavoenzymes – high similarity of molecular properties emphasizes their central role in sulfur metabolism. FEBS Letters **473**:63-66.

30. **Gonzalez, C. F., A. J. Stonestrom, G. L. Lorca, and M. H. Saier.** 2005. Biochemical characterization of phosphoryl transfer involving HPr of the phosphoenolpyruvate-dependent phosphotransferase system in *Treponema denticola*, an organism that lacks PTS permeases. Biochemistry **44**:598-608.

31. **Gordon, D., C. Abajian, and P. Green.** 1998. Consed: a graphical tool for sequence finishing. Genome Research **8**:195-202.

32. **Grass, G., and C. Rensing.** 2001. CueO is a multi-copper oxidase that confers copper tolerance in *Escherichia coli*. Biochem. Biophys. Res. Commun. **286**:902-908.

33. **Hayashi, N. R., H. Arai, T. Kodama, and Y. Igarashi.** 1997. The novel genes, *cbb*Q and *cbb*O, located downstream from the RubisCO genes of *Pseudomonas hydrogenothermophila*, affect the conformational states and activity of RubisCO. Biochem. Biophys. Res. Commun. **241**: 565–569.

34. **Heidelberg, J. F., I. T. Paulsen, K. E. Nelson, E. J. Gaidos, W. C. Nelson, T. D. Read, J. A. Eisen, R. Seshadri, N. Ward, B. Methe, R. A. Clayton, T. Meyer, A. Tsapin, J. Scott, M. Beanan, L. Brinkac, S. Daugherty, R. T. DeBoy, R. J. Dodson, A. S. Durkin, D. H. Haft, J. F. Kolonay, R. Madupu, J. D. Peterson, L. A. Umayam, O. White, A. M. Wolf, J. Vamathevan, J. Weidman, M. Impraim, K. Lee, K. Berry, C. Lee, J. Mueller, H. Khouri, J. Gill, T. R. Utterback, L. A. McDonald, T. V. Feldblyum, H. O. Smith, J. C. Venter, K. H. Nealson, and C. M. Fraser.** 2002. Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*. Nature Biotechnol. **20**: 1118-1123.

35. **Hernandez, J. M., S. H. Baker, S. C. Lorbach, J. M. Shively, and F. R. Tabita.** 1996. Deduced amino acid sequence, functional expression, and unique enzymatic properties of the Form I and Form II ribulose bisphosphate carboxylase/oxygenase from the chemoautotrophic bacterium *Thiobacillus denitrificans*. J. Bacteriol. **178**: 347-356.

36. **Hole, U. H., K.-U. Vollack, W. G. Zumft, E. Eisenmann, R. A. Siddiqui, B. Friedrich, P. M. H. Kroneck.** 1996. Characterization of the membranous denitrification enzymes nitrite reductase (cytochrome cd_1) and copper-containing nitrous oxide reductase from *Thiobacillus denitrificans*. Arch. Microbiol. **165**:55-61.

37. **Hommes, N. G., L. A. Sayavedra-Soto, and D. J. Arp.** 2003. Chemolithoorganotrophic growth of *Nitrosomonas europaea* on fructose. J. Bacteriol. **185**: 6809-6814.

38. **Jeanmougin, F., J. D. Thompson, M. Gouy, D. G. Higgins, and T. J. Gibson.** 1998. Multiple sequence alignment with Clustal X. Trends Biochem Sci. **23**:403-5.

39. **Kappler, U., C. G. Friedrich, H. G. Trüper, and C. Dahl.** 2001. Evidence for two pathways of thiosulfate oxidation in *Starkeya novella* (formerly *Thiobacillus novellus*). Arch. Microbiol. **175**:102-111.

40. **Karlin S., J. Mrazek, and A. M. Campbell.** 1997. Compositional biases of bacterial genomes and evolutionary implications. J. Bacteriol. **179**:3899-3913.

41. **Kelly, D. P.** 1967. The incorporation of acetate by the chemoautotroph *Thiobacillus neapolitanus* strain C. Arch. Mikrobiol. **58**: 99-116.

42. **Kelly, D. P.** 1982. Biochemistry of the chemolithotrophic oxidation of inorganic sulphur. Phil. Trans. Roy. Soc. London **B298**:499-528.

43. **Kelly, D. P.** 1989. Physiology and biochemistry of unicellular sulfur bacteria, p. 193-217. *In* H. G. Schlegel and B. Bowien (ed.), Biology of autotrophic bacteria, Science Tech Publishers, Madison, Wisconsin.

44. **Kelly, D. P.** 1990. Energetics of chemolithotrophic bacteria, p. 479-503. *In* T. A. Krulwich (ed.), Bacterial energetics, Academic Press, San Diego.

45. **Kelly, D. P.** 1999. Thermodynamic aspects of energy conservation by chemolithotrophic sulfur bacteria in relation to the sulfur oxidation pathways. Arch. Microbiol. **171**:219-229.

46. **Kelly, D. P., and A. P. Harrison.** 1989. Genus *Thiobacillus*, p. 1842-1858. *In* J. T. Staley, M. P. Bryant, N. Pfennig, and J. G. Holt (ed.), Bergey's Manual of Systematic Bacteriology, 1st edition, vol. 3. Williams & Wilkins, Baltimore.

47. **Kelly, D. P., and A. P. Wood.** 2000. Confirmation of *Thiobacillus denitrificans* as a species of the genus *Thiobacillus*, in the beta-subclass of the *Proteobacteria*, with strain NCIMB 9548 as the type strain. Int. J. Syst. Evol. Microbiol. **50**:547-550.

48. **Kelly, D. P., and A. P. Wood.** 2000. The chemolithotrophic prokaryotes. *In* The prokaryotes, an evolving electronic resource for the microbiological community (PKIIIE), M. Dworkin, Falkow S, Rosenberg E, Schleifer K-H, and E. Stackebrandt. Springer-Verlag, New York: (http://www.prokaryotes.com).

49. **Kelly, D. P., A. P. Wood, and E. Stackebrandt.** 2005. Genus II. *Thiobacillus* Beijerinck, p. 764-769. *In* Bergey's Manual of Systematic Bacteriology, 2nd edition, vol. 2, part C, Springer, New York.

50. **Kelly, D. P., J. K. Shergill, W-P. Lu, and A. P. Wood.** 1997. Oxidative metabolism of inorganic sulfur compounds by bacteria. Antonie van Leeuwenhoek **71**:95-107.

51. **Kobayashi, I.** 2001. Behavior of restriction-modification systems as selfish mobile elements and their impact on genome evolution. Nucleic Acids Res. **29**:3742-56.

52. **Krogh, A., B. Larsson, G. von Heijne, and E. L. L. Sonnhammer.** 2001. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. J. Mol. Biol. **305**:567-580.

53. **Laska, S., F. Lottspeich, and A. Kletzin.** 2003. Membrane-bound hydrogenase and sulfur reductase of the hyperthermophilic and acidophilic archaeon *Acidianus ambivalens*. Microbiology **149**:2357-2371.

54. **Methé, B. A., K. E. Nelson, J. A. Eisen, I. T. Paulsen, W. Nelson, J. F. Heidelberg, D. Wu, M. Wu, N. Ward, M. J. Beanan, R. J. Dodson, R. Madupu, L. M. Brinkac, S. C. Daugherty, R. T. DeBoy, A. S. Durkin, M. Gwinn, J. F. Kolonay, S. A. Sullivan, D. H. Haft, J. Selengut, T. M. Davidsen, N. Zafar, O. White, B. Tran, C. Romero, H. A. Forberger, J. Weidman, H. Khouri, T. V. Feldblyum, T. R. Utterback, S. E. Van Aken, D. R. Lovley, and C. M. Fraser.** 2003. Genome of *Geobacter sulfurreducens*: metal reduction in subsurface environments. Science **302**: 1967-1969.

55. **Nies, D. H.** 2003. Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol. Rev. **27**:313-339.

56. **Pauwels, H., W. Kloppmann, J.-C. Foucher, A. Martelat, and V. Fritsche.** 1998. Field tracer test for denitrification in a pyrite-bearing schist aquifer. Appl. Geochem. **13**: 767-778.

57. **Peeters, T. L., M. S. Liu, and M. I. H. Aleem.** 1970. The tricarboxylic acid cycle in *Thiobacillus denitrificans* and *Thiobacillus-A2*. J. Gen. Microbiol. **64**: 29-35.

58. **Pereira, M. M., and M. Teixeira.** 2004. Proton pathways, ligand binding and dynamics of the catalytic site in haem-copper oxygen reductases: a comparison between the three families. Biochim. Biophys. Acta **1655**:340–346.

59. **Petri, R., L. Podgorsek, and J. F. Imhoff.** 2001. Phylogeny and distribution of the *soxB* gene among thiosulfate-oxidizing bacteria. FEMS Microbiol. Lett. **197**:171-178.

60. **Philippot, L.** 2002. Denitrifying genes in bacterial and Archaeal genomes. Biochim. Biophys. Acta **1577**:355-376.

61. **Pitcher, R. S., and N. J. Watmough.** 2004. The bacterial cytochrome *cbb$_3$* oxidases. Biochim. Biophys. Acta **1655**:388-399.

62. **Pott, A. S., and C. Dahl.** 1998. Sirohaem sulfite reductase and other proteins encoded by genes at the *dsr* locus of *Chromatium vinosum* are involved in the oxidation of intracellular sulfur. Microbiology, UK **144**:1881-1894.

63. **Quentmeier, A., P. Hellwig, F. Bardichewsky, G. Grolle, R. Kraft, and C. G. Friedrich.** 2003. Sulfur oxidation in *Paracoccus pantotrophus*: interaction of the sulfur-binding protein SoxYZ with the dimanganese SoxB protein. Biochem. Biophys. Res. Commun. **312**:1011-1018.

64. **Quentmeier, A., P. Hellwig, F. Bardichewsky, R. Wichmann, C. G. Friedrich.** 2004. Sulfide dehydrogenase activity of the monomeric flavoprotein SoxF of *Paracoccus pantotrophus*. Biochemistry **43**:14696-14703.

65. **Quentmeier, A., R. Kraft, S. Kostka, R. Klockemkaper, and C. G. Friedrich.** 2000. Characterization of a new type of sulfite dehydrogenase from *Paracoccus pantotrophus* GB17. Arch. Microbiol. **173**:117-125.

66. **Rakhely, G., A. Colbeau, J. Garin, P. M. Vignais, and K. L. Kovacs.** 1998. Unusual organization of the genes coding for HydSL, the stable [NiFe]hydrogenase in the photosynthetic bacterium *Thiocapsa roseopersicina*. J. Bacteriol. **180**:1460-1465.

67. **Rother, D., and C. G. Friedrich.** 2002. The cytochrome complex SoxXA of *Paracoccus pantotrophus* is produced in *Escherichia coli* and functional in the reconstituted sulfur-oxidizing enzyme system. Biochim. Biophys. Acta **1598**:65-73.

68. **Rother, D., H. J. Henrich, A. Quentmeier, F. Bardichewsky, and C. G. Friedrich. 2001**. Novel genes of the *sox* gene cluster, mutagenesis of the flavoprotein SoxF, and evidence for a general sulfur-oxidizing system in *Paracoccus pantotrophus* GB17. J. Bacteriol. **183**:4499-4508.

69. **Sawnhey, V., and D. J. D. Nicholas**. 1977. Sulphite-dependent nitrate reductase and NADH-dependent nitrate reductase from *Thiobacillus denitrificans*. J. Gen. Microbiol. **100**:49-58.

70. **Sawnhey, V., and D. J. D. Nicholas**. 1978. Sulphide-linked nitrite reductase from *Thiobacillus denitrificans* with cytochrome oxidase activity: purification and properties. J. Gen. Microbiol. **106**:119-128.

71. **Schedel, M. and H. G. Trüper.** 1979. Purification of *Thiobacillus-denitrificans* siroheme sulfite reductase and investigation of some molecular and catalytic properties. Biochim. Biophys. Acta **568**:454-466.

72. **Shively, J. M., G. L. Decker, and J. W. Greenwalt.** 1970. Comparative ultrastructure of the thiobacilli. J. Bacteriol. **96**:2138–2143.

73. **Smith, A. J., and D. S. Hoare.** 1977. Specialist phototrophs, lithotrophs, and methylotrophs: a unity among a diversity of prokaryotes? Bacteriol. Rev. **41**: 419-448.

74. **So, A. K.-C., G. S. Espie, E. B. Williams, J. M. Shively, S. Heinhorst, and G. C. Cannon.** 2004. A novel evolutionary lineage of carbonic anhydrase (ε class) is a component of the carboxysome shell. J. Bacteriol. **186**: 623-630.

75. **Sonnhammer, E. L. L., G. von Heijne, and A. Krogh.** 1998. A hidden Markov model for predicting transmembrane helices in protein sequences, p. 175-182. *In* J. Glasgow, T. Littlejohn, F. Major, R. Lathrop, D. Sankoff, and C. Sensen (ed.), Proceedings of the Sixth International Conference on Intelligent Systems for Molecular Biology, AAAI Press, Menlo Park, CA.

76. **Sorokin, D. Y., T. P. Tourova, A. N. Antipov, G. Muyzer, and J. G. Kuenen.** 2004. Anaerobic growth of the haloalkaliphilic denitrifying sulfur-oxidizing bacterium *Thialkalivibrio thiocyanodenitrificans* sp. nov. with thiocyanate. Microbiology, UK **150**:2435-2442.

77. **Stover, C. K., X. Q. Pham, A. L. Erwin, S. D. Mizoguchi, P. Warrener, M. J. Hickey, F. S. L. Brinkman, W. O. Hufnagle, D. J. Kowalik, M. Lagrou, R. L. Garber, L. Goltry, E. Tolentino, S. Westbrock-Wadman, Y. Yuan, L. L. Brody, S. N. Coulter, K. R. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G. K.-S. Wong, Z. Wu, I. T. Paulsen, J. Reizer, M. H. Saier, R. E. W. Hancock, S. Lory, and M. V. Olson.** 2000. Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen. Nature **406**:959-964.

78. **Straub, K. L., M. Benz, B. Schink, and F. Widdel.** 1996. Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. Appl. Environ. Microbiol. **62**:1458-1460.

79. **Stubner S., T. Wind, and R. Conrad.** 1998. Sulfur oxidation in rice field soil: activity, enumeration, isolation and characterization of thiosulfate-oxidizing bacteria. Syst. Appl. Microbiol. **21**: 569-578.

80. **Suzuki, Y., S. D. Kelly, K. M. Kemner, and J. F. Banfield.** 2003. Microbial populations stimulated for hexavalent uranium reduction in uranium mine sediment. Appl. Environ. Microbiol. **69**:1337-1346.

81. **Tanabe, T., T. Funahashi, H. Nakao, S. Miyoshi, S. Shinoda, and S. Yamamoto.** 2003. Identification and characterization of genes required for biosynthesis and transport of the siderophore vibrioferrin in *Vibrio parahaemolyticus*. J. Bacteriol. **185**: 6938-6949.

82. **Tatusov, R. L., M. Y. Galperin, D. A. Natale, and E. V. Koonin.** 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res. **28**:33-36.

83. **Taylor, B. F., and D. S. Hoare.** 1971. *Thiobacillus denitrificans* as an obligate autotroph. Cell suspension and enzymic studies. Arch. Mikrobiol. **80**: 262-276.

84. **Taylor, B. F., D. S. Hoare, and S. L. Hoare.** 1971. *Thiobacillus denitrificans* as an obligate autotroph. Isolation and growth studies. Arch. Mikrobiol. **78**: 193-204.

85. **Timmer-Ten Hoor, A.** 1975. A new type of thiosulfate oxidizing, nitrate reducing microorganism: *Thiomicrospira denitrificans* sp. nov. Netherlands J. of Sea Research **9**:344-351.

86. **Trüper, H. G.** 1994. Reverse siroheme sulfite reductase from *Thiobacillus denitrificans*. Meth. Enzymol. **243**: 422-426.

87. **Vignais, P. M., B. Billoud, and J. Meyer.** 2001. Classification and phylogeny of hydrogenases. FEMS Microbiol. Rev. **25**: 455-501.

88. **Vlasceanu, L., R. Popa, and B. K. Kinkle.** 1999. Characterization of *Thiobacillus thioparus* LV43 and its distribution in a chemoautotrophically based groundwater ecosystem. Appl. Environ. Microbiol. **63**: 3123-3127.

89. **Ward N., O. Larsen, J. Sakwa, L. Bruseth, H. Khouri, A. S. Durkin, G. Dimitrov, L. X. Jiang, D. Scanlan, K. H. Kang, M. Lewis, K. E. Nelson, B. Methé, M. Wu, J. F. Heidelberg, I. T. Paulsen, D. Fouts, J. Ravel, H. Tettelin, Q. Ren, T. Read, R. T. DeBoy, R. Seshadri, S. L. Salzberg, H. B. Jensen, N. K. Birkeland, W. C. Nelson, R. J. Dodson, S. H. Grindhaug, I. Holt, I. Eidhammer, I. Jonasen, S. Vanaken, T. Utterback, T. V. Feldblyum, C. M. Fraser, J. R. Lillehaug, and J. A. Eisen.** 2004. Genomic insights into methanotrophy: the complete genome sequence of *Methylococcus capsulatus* (Bath). PLoS Biology **2**: e303 (http://www.plosbiology.com).

90. **Whittenbury R., and D. P. Kelly.** 1977. Autotrophy: a conceptual phoenix. Symp. Soc. Gen. Microbiol. **27**: 121-149.

91. **Wodara, C., S. Kostka, M. Egert, D. P. Kelly, and C. G. Friedrich**. 1994. Identification and sequence analysis of the *soxB* gene essential for sulfur oxidation of *Paracoccus denitrificans* GB17. J. Bacteriol. **176**:6188-6191.

92. **Wood, A. P., J. P. Aurikko, and D. P. Kelly.** 2004. A challenge for 21st century molecular biology and biochemistry: what are the causes of obligate autotrophy and methanotrophy? FEMS Microbiol. Rev. **28**: 335-352.

93. **Zaman, Z., S. B. Bowman, G. D. Kornfeld, A. J. Brown, and I. W. Dawes.** 1999. Transcription factor GCN4 for control of amino acid biosynthesis also regulates the expression of the gene for lipoamide dehydrogenase. Biochem. J. **340**: 855-862.

94. **Zumft, W. G.** 1997. Cell biology and molecular basis of denitrification. Microbiol. Mol. Biol. Rev. **61**:533-616.

TABLE 1. General features of the *T. denitrificans* strain ATCC 25259 genome.

Chromosome size, base pairs	2,909,809
G+C ratio	66.07 %
Coding density	92.5 %
Number of predicted protein coding genes	2827
Average CDS length, bp	952
Number of predicted proteins unique to *T. denitrificans* (%)	89 (3.1%)
rRNA operons	2
tRNA genes	43
Other small RNAs	3
Number of predicted proteins with putative function (%)	2183 (77.2%)
Number of predicted proteins with unknown function (%)	644 (22.8%)
Protein categories (%)	
Energy production	6.6 %
Inorganic ion transport	5.7 %
Cell envelope biogenesis	6.0 %
BLASTP comparison against the KEGG completed microbial genomes database (number of top KEGG hits)	
β-proteobacteria	1624
γ-proteobacteria	455
α-proteobacteria	119
δ-proteobacteria	92
Archaea	22

TABLE 2. Regions with uncharacteristic G+C content and Karlin signatures[a].

Location in genome	% G+C	CDS	BLAST hits
394058..402433	58.87	Tbd0363-0373	Phage integrase, regulatory protein, hypothetical proteins
979890..1004773	57.39	Tbd0925-0943	Methylase, transposase, regulatory protein, helicase (Snf2/Rad54 family), type III restriction/modification system (methylase, restriction enzyme), phage integrases and several hypothetical proteins
1264595..1271704	56.99	Tbd1207-1215	Phage integrase, conserved imported protein, and hypothetical proteins
1395068..1400129	52.43	Tbd1314-1318	Phage integrase, and hypothetical and conserved hypothetical proteins
1424272..1431083	60.88	Tbd1342-1354	Mostly hypothetical proteins, and a conserved imported protein
1575347..1583813	57.71	1487-1492	Type II/III restriction/modification system (helicase, methylase), conserved hypothetical and hypothetical proteins

1767440..1787406 [b]	60.46	Tbd1679-1695	Phage integrase, conjugal transfer region (TraWBAY), a number of hypothetical proteins, and imported AAA superfamily ATPase and conserved hypothetical protein
1955614..1961331	61.96	Tbd1861-1865	Type IV pilus proteins (PilEWXV and FimT)
2052544..2061073	59.64	Tbd1958-1969	Transposases, conserved imported hypothetical proteins and hypothetical proteins
2089032..2093604 [b]	57.82	Tbd2000-2005	Phage integrase, plasmid recombination protein, and several hypothetical proteins
2160137..2166983 [b]	62.36	Tbd2066-2073	Phage integrase, phage replication protein, DNA helicase and several hypothetical proteins
2750091..2764591	57.46	Tbd2675-2685	Phage integrase, prophage regulatory protein, Type I restriction/modification system (HsdMS, R)
2900270..2905890	61.02	Tbd2817-2823	Phage integrase, phage primase and phage regulatory protein

[a] All regions are also supported by Karlin signature difference.

[b] Flanked by a tRNA.

TABLE 3. Differential transcription of selected genes in *T. denitrificans* that occur in multiple copies

Genes compared	Thiosulfate-induced[a] (fold difference)	$FeCO_3$-induced[b] (fold difference)
dsrC (putative)		
Tbd2480/Tbd2480[c]	1[d]	0.38
Tbd2488/Tbd2480[c]	1.4	0.025
Tbd2658/Tbd2480[c]	0.069	0.006
Tbd2326/Tbd2480[c]	0.015	0.002
Tbd2327/Tbd2480[c]	0.009	0.002
Tbd1365/Tbd2480[c]	0.039	0.009
Tbd1408/Tbd2480[c]	0.82	0.031
Tbd1926/Tbd2480[c]	0.001	0.002
Adenylylsulfate reductase		
α subunit		
Tbd0872/Tbd2282	80	NA[e]
β subunit		
Tbd0873/Tbd2283	740	NA
ATP sulfurylase		
Tbd0874/Tbd0210	5	NA
Nitric oxide reductase		
norC		
Tbd0562/Tbd0822	63	NA
norB		

Tbd0561/Tbd0823	61	NA

[a] Cells harvested for RNA while carrying out thiosulfate oxidation and denitrification (see Materials and Methods).

[b] Cells harvested for RNA while carrying out oxidation of Fe(II) (in $FeCO_3$) and denitrification (see Materials and Methods).

[c] Comparison made to the number of transcripts for Tbd2480 under thiosulfate-induced conditions. The transcript copy number for Tbd2480 under these conditions was relatively high (in the copy number range of Tbd0562 and 0561, which encode subunits of nitric oxide reductase, a key enzyme involved in denitrification).

[d] By definition.

[e] Not analyzed.

TABLE 4. CDS potentially encoding *c*-type cytochromes in the *T. denitrificans* genome[a]

CDS	Annotation[b]	Molecular mass (Da)[c]	No. of hemes
Tbd0055	Cytochrome *c* family protein	20116	1
Tbd0064	Cytochrome *c*-553	23727	2
Tbd0070	Probable *nirN*	63589	1
Tbd0076	Probable *nirC*	10547	1
Tbd0077	*nirS* (cytochrome cd_1)	62992	1
Tbd0094	Hypothetical protein	17109	1
Tbd0128	Cytochrome *c*	38000	2
Tbd0129	Cytochrome *c*	21784	2
Tbd0137	Diheme cytochrome *c*	19427	2
Tbd0138	Cytochrome *c*-type protein	14395	1
Tbd0146	Probable cytochrome *c*5	26734	2
Tbd0187	Cytochrome *c*	21387	2
Tbd0219	FAD/FMN-containing dehydrogenase	138251	1
Tbd0325	aa_3-type cytochrome *c* oxidase, subunit II	41279	1
Tbd0339	cbb_3-type cytochrome *c* oxidase, subunit II	28037	1
Tbd0341	cbb_3-type cytochrome *c* oxidase, subunit III	33124	2
Tbd0436	Excinuclease ATPase subunit	103145	1
Tbd0562	*norC*	15845	1
Tbd0564	*soxA*	30960	1
Tbd0567	*soxX*	12712	1
Tbd0571	DMSO reductase chain B	25909	1

Tbd0640	*cbb*$_3$-type cytochrome *c* oxidase, subunit III	33873	2
Tbd0642	*cbb*$_3$-type cytochrome *c* oxidase, subunit II	22314	1
Tbd0723	Possible high-affinity Fe^{2+}/Pb^{2+} permease	69511	1
Tbd0752	MSHA pilin biogenesis ATPase protein MshE	62481	1
Tbd0820	Cytochrome *c* (in/near nonfunctional *nor* cluster)	57261	2
Tbd0822	*norC*-related (potentially not functional)	30200	2
Tbd0840	Probable cytochrome c_5	16718	1
Tbd0917	*soxX*	13246	1
Tbd0918	*soxA*	30875	1
Tbd1169	Ferredoxin, 2Fe-2S	12259	1
Tbd1357	Unknown	16635	1
Tbd1398	Putative cytochrome *c*-type protein	15842	1
Tbd1404	*narH*	59191	1
Tbd1484	Cytochrome *c*	9544	1
Tbd1520	Putative Fe-S protein	48430	1
Tbd1542	ATPase involved in DNA replication	61297	1
Tbd1564	Probable ribonuclease E	95354	1
Tbd1585	Putative pyruvate formate-lyase-activating enzyme	40800	1
Tbd1831	Putative cytochrome c_1	27211	1
Tbd1840	Unknown	11061	1
Tbd2026	Possible cytochrome c_4 or *c*-553	11253	1
Tbd2027	Cytochrome c, class IC	11521	1
Tbd2034	Possible cytochrome subunit of sulfide dehydrogenase	10395	1

Tbd2060	Possible alpha-mannosidase	64835	1
Tbd2157	Cytochrome *c*	18742	1
Tbd2170	Activase of anaerobic class III ribonucleotide reductase	24585	1
Tbd2181	Unknown	20904	1
Tbd2476	*dsrJ*	17948	3
Tbd2477	*dsrL*	71328	1
Tbd2545	Diheme cytochrome *c*	37973	2
Tbd2726	Cytochrome *c*	11096	1
Tbd2727	Conserved protein of unknown function	74616	1
Tbd2738	Zinc-dependent hydrolase	26287	1

[a] As defined by the presence of at least one CXXCH heme-binding motif. Tbd0039 (which encodes ribosomal protein L31) and Tbd1539 (which encodes DnaJ) include the CXXCH motif but were excluded from this table.

[b] Best attempt at annotation based on examination of best BLASTP hits, top ten PSI-BLAST hits, and genomic context.

[c] Molecular mass predicted for the unprocessed gene product without cofactors.

TABLE 5. Comparison of encoded efflux-mediated heavy metal resistance systems among the genomes of *T. denitrificans* and other selected bacteria.

Bacterial species	Genome size (Mbp)	HME RND	CDF	CPx-type ATPases
Thiobacillus denitrificans[a]	2.9	5	3	3
Geobacter sulfurreducens PCA[a]	3.8	3	2	2
Ralstonia metallidurans[b]	6.9	12	3	5
Pseudomonas aeruginosa[b]	6.3	1	3	4
Escherichia coli[b]	4.6	1	2	2

[a]Putative heavy metal-exporting protein families for *T. denitrificans* and *G. sulfurreducens* PCA (GenBank AE01780) were identified as described by Nies (55). Confirmatory data on metal transport in *G. sulfurreducens* was obtained from an unpublished source (H. A. Vrionis and D. R. Lovley, 2005, Poster I-054 at the ASM 105th General Meeting).

[b]From Nies (55).

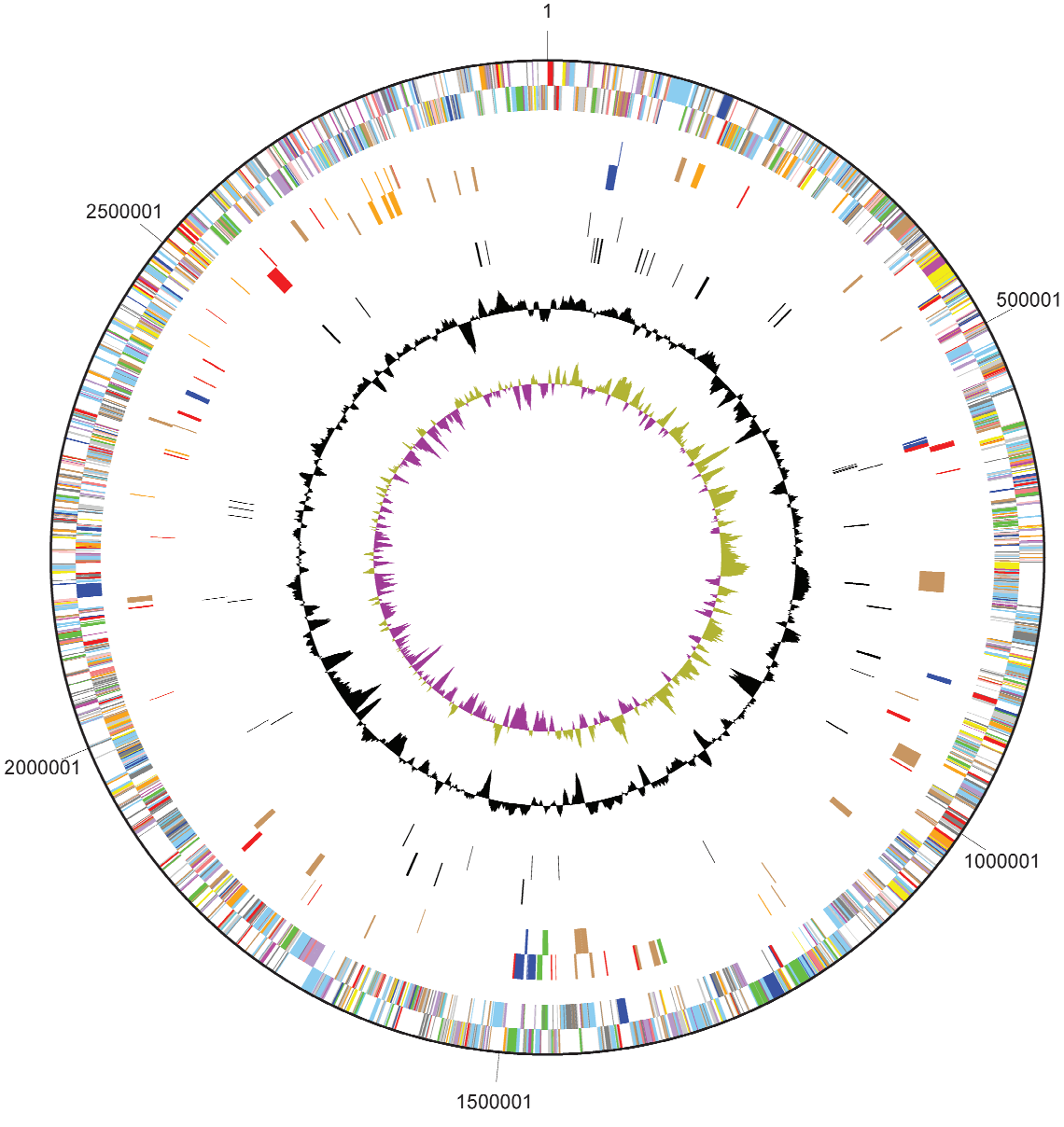
FIGURE LEGENDS

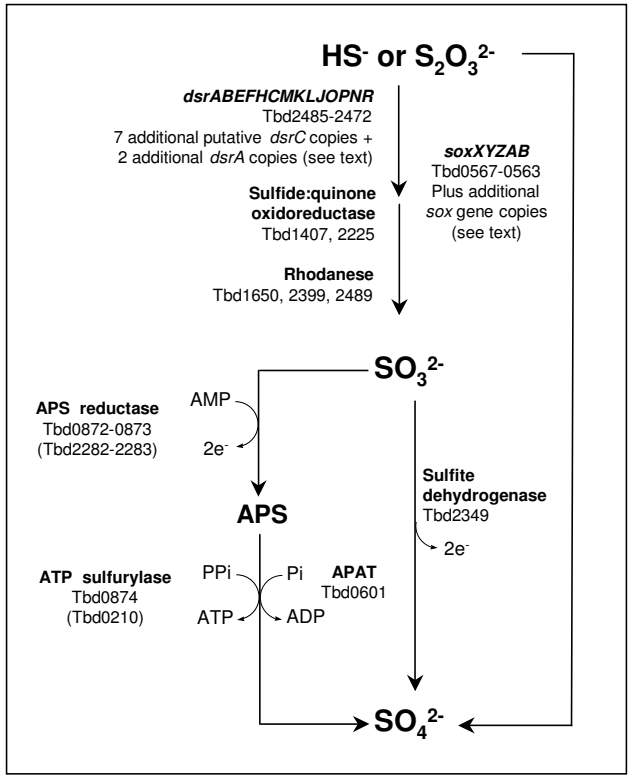
FIG. 1. Schematic circular diagram of the *T. denitrificans* ATCC 25259 genome. Outer circle, predicted coding regions on the forward strand, color-coded by role categories (dark grey - hypothetical proteins, light grey- conserved hypothetical and unknown function, brown - general function, red – DNA replication and repair, green - energy metabolism, blue - carbon and carbohydrate metabolism, cyan - lipid metabolism, magenta - transcription, yellow - translation, orange - amino acid metabolism, pink - metabolism of cofactors and vitamins, light red - purine and pyrimidine metabolism, lavender - signal transduction, sky blue - cellular processes, pale green - structural RNAs). Second circle, predicted coding regions on the reverse strand, color-coded as for outer circle. Third and fourth circles, coding regions (on forward and reverse strands) predicted to be involved in denitrification (blue), sulfur-compound oxidation (red), hydrogen oxidation (green), autotrophic carbon assimilation (orange), and metal ion transport/resistance (brown). Fifth and sixth circles, coding regions found to have a CXXCH heme-binding motif and therefore potentially encoding *c*-type cytochromes. Seventh circle, deviation from the average G+C. Eighth circle, GC skew (positive – olive; negative – purple).

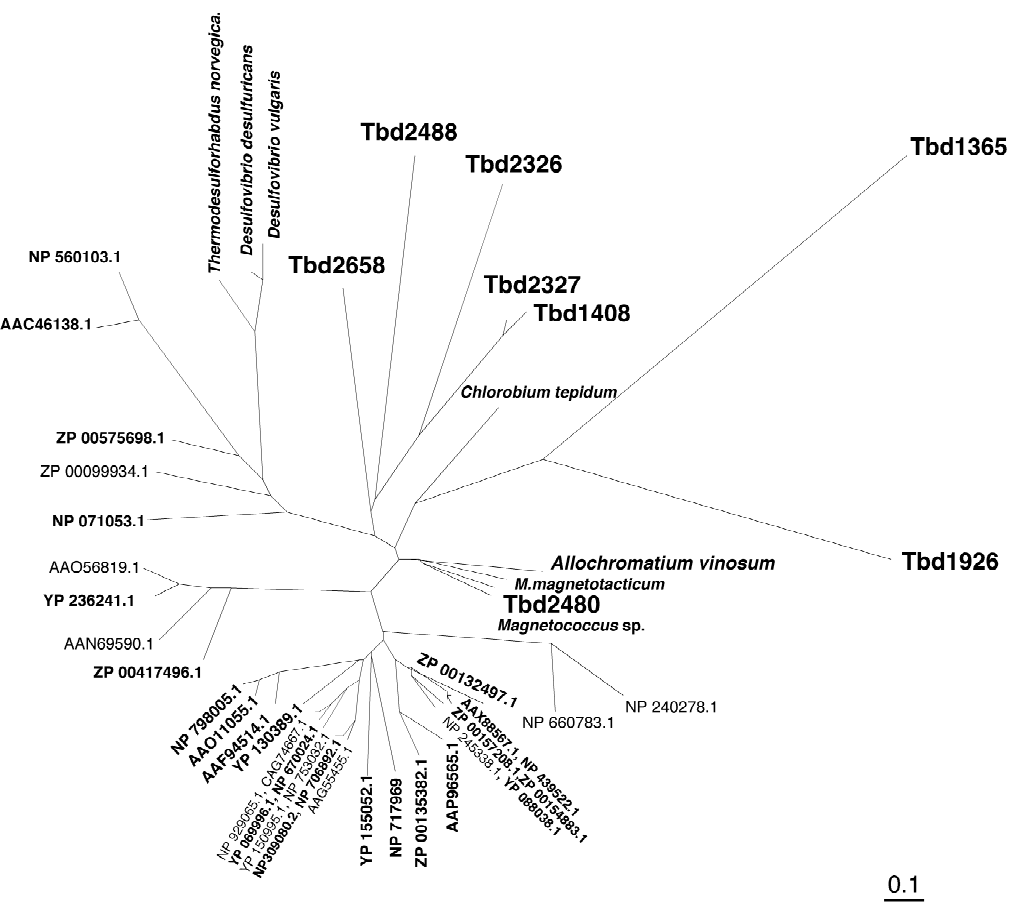
FIG. 2. Schematic overview of key genes/enzymes putatively associated with sulfur-compound oxidation in *T. denitrificans*. Genes in parenthesis have been shown to be lesser expressed paralogs (this study). The biochemical roles of a number of gene products represented in this figure have not been experimentally demonstrated in *T. denitrificans* and are uncertain. Sulfide:quinone oxidoreductase is not proposed to catalyze the direct oxidation of sulfide to sulfite, but rather may participate in an indirect pathway (20). The arrow between thiosulfate and sulfate (right side) represents the possibility that SoxB catalyzes a sulfate thiohydrolase reaction (28) in *T. denitrificans*.

FIG. 3. Phylogenetic relationships among the eight putative DsrC proteins encoded in *T. denitrificans* ATCC 25259 and the top BLASTP matches from the GenBank nr database for Tbd2480. Of the proteins represented in this figure that are not from *T. denitrificans*, more than 70 percent are known or predicted to be DsrC or more broadly related to sulfite reductases (indicated in bold faced type). For limbs that show species names rather than GenBank accession numbers, the corresponding accession numbers are as follows: *A. vinosum* (AAC35399.1), *M. magnetotacticum* (ZP 00052645.1), *Magnetococcus* sp. (ZP 00287929.1), *C. tepidum* (NP 663123.1), *T. norvegica* (CAC36215.1), *D. desulfuricans* (ZP 00130056.2), and *D. vulgaris* (YP 011988).

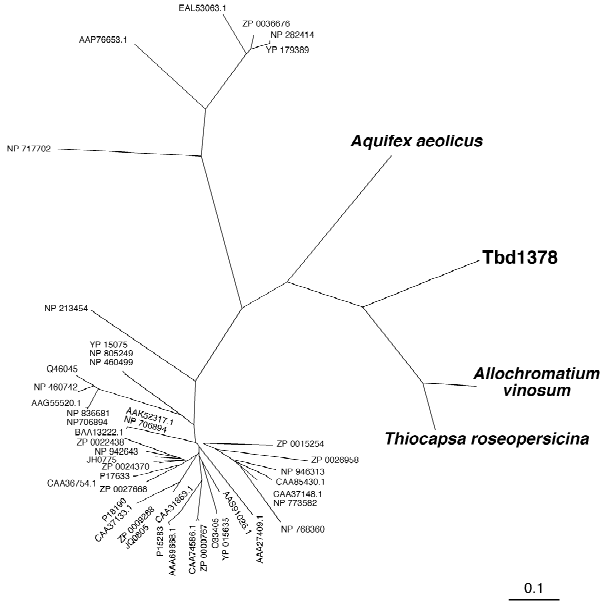
FIG. 4. Phylogenetic relationships among predicted amino acid sequences for HynS (A), Isp1 (B), Isp2 (C), and HynL (D) in *T. denitrificans* and the best BLASTP matches from the GenBank nr database. For limbs that show species names rather than GenBank accession numbers, the corresponding accession numbers are as follows: (HynS) *A. vinosum* (AAU93828.1), *T. roseopersicina* (AAC38281.1), *A. aeolicus* (NP 213658.1); (Isp1) *A. vinosum* (AAU93829.2), *T. roseopersicina* (AAC38283.1), *A. aeolicus* (NP 213657.1), *D. vulgaris* Hmc5 (YP 009755), *A. ambivalens* (CAC86885.1), *D. desulfuricans* NarI (ZP 00128546.1); (Isp2) *A. vinosum* (AAY89333.1), *T. roseopersicina* (AAC38284.1), *A. aeolicus* (NP 213656.1), *A. ambivalens* (CAC86886.1), *Polaromonas* sp. (ZP 00503323.1), *C. aurantiacus* (ZP 00356812), *A. vinosum* DsrK (AAC35401.2), *C. tepidum* DsrK (NP 663117.1), *D. vulgaris* Hmc6 (YP 009754.1); (HynL) *A. vinosum* (AAY89334.1), *T. roseopersicina* (AAC38282.1), *A. aeolicus* (NP 213655.1).


1
500001
1000001
1500001
2000001
2500001

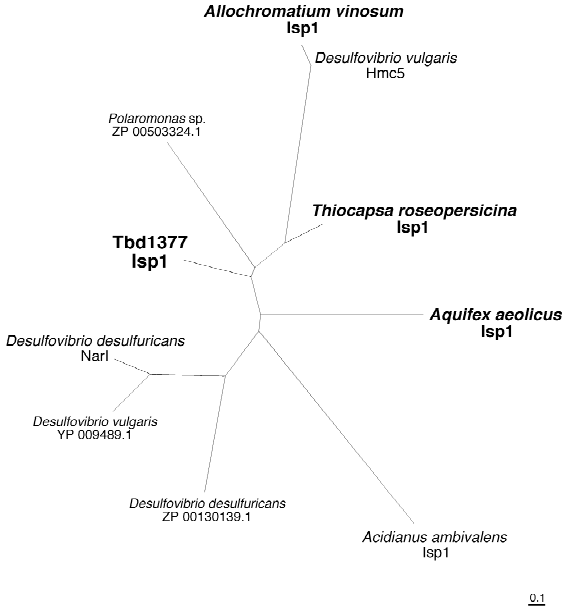

HS- or S2O32-
dsrABEFHCMKLJOPNR
Tbd2485-2472
7 additional putative dsrC copies +
2 additional dsrA copies (see text)
soxXYZAB
Tbd0567-0563
Plus additional
sox gene copies
(see text)
Sulfide:quinone
oxidoreductase
Tbd1407, 2225
Rhodanese
Tbd1650, 2399, 2489
SO32-
APS reductase
Tbd0872-0873
(Tbd2282-2283)
AMP
2e-
APS
Sulfite
dehydrogenase
Tbd2349
2e-
ATP sulfurylase
Tbd0874
(Tbd0210)
PPi
Pi
APAT
Tbd0601
ATP
ADP
SO42-


Thermodesulforhabdus norvegica.
Desulfovibrio desulfuricans
Desulfovibrio vulgaris
Tbd2488
Tbd2326
Tbd1365
NP 560103.1
Tbd2658
Tbd2327
Tbd1408
AAC46138.1
Chloroblum tepidum
ZP 00575698.1
ZP 00099934.1
NP 071053.1
Tbd1926
AAO56819.1
Allochromatium vinosum
M.magnetotacticum
YP 236241.1
Tbd2480
Magnetococcus sp.
AAN69590.1
ZP 00417496.1
ZP 00132497.1
NP 798005.1
AAO11055.1
AAF94514.1
YP 130389.1
NP 660783.1
NP 240278.1
AAX88567.1
NP 439522.1
ZP 00157208.1
ZP 00151983.1
YP 080038.1
NP 870024.1
NP 706992.1
AAO55435.1
YP 155052.1
NP 717969
ZP 00135382.1
AAP96565.1
0.1

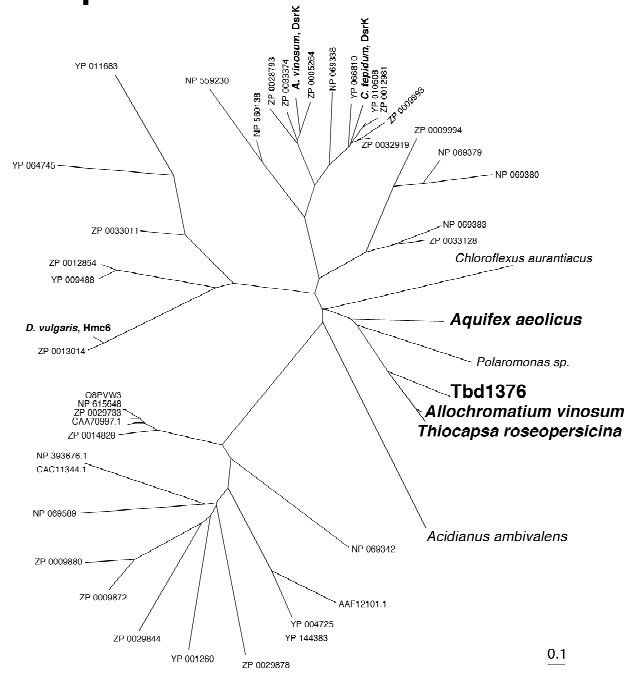
A. HynS



B. Isp1



C. Isp2



D. HynL