# Chapter 17

# Genomic Insights into the Sulfur Metabolism of Phototrophic Green Sulfur Bacteria

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*Rüdiger Hell et al. (eds.), Sulfur Metabolism in Phototrophic Organisms,* 337–355.

## Summary

Green sulfur bacteria (GSB) utilize various combinations of sulfide, elemental sulfur, thiosulfate, ferrous iron, and hydrogen for anaerobic photoautotrophic growth. Genome sequence data is currently available for 12 strains of GSB. We present here a genome-based survey of the distribution and phylogenies of genes involved in oxidation of sulfur compounds in these strains. Sulfide:quinone reductase, encoded by *sqr*, is the only known sulfur-oxidizing enzyme found in all strains. All sulfide-utilizing strains contain the dissimilatory sulfite reductase *dsrABCEFHLNMKJOPT* genes, which appear to be involved in elemental sulfur utilization. All thiosulfate-utilizing strains have an identical *sox* gene cluster (*soxJXYZAKBW*). The *soxCD* genes found in certain other thiosulfate-utilizing organisms like *Paracoccus pantotrophus* are absent from GSB. Genes encoding flavocytochrome *c* (*fccAB*), adenosine-5′-phosphosulfate reductase (*aprAB*), ATP-sulfurylase (*sat*), a homolog of heterodisulfide reductase (*qmoABC*), and other enzymes related to sulfur utilization are found in some, but not all sulfide-utilizing strains. Other than *sqr, Chlorobium ferrooxidans*, a $Fe^{2+}$-oxidizing organism that cannot grow on sulfide, has no genes obviously involved in oxidation of sulfur compounds. Instead, *Chl. ferrooxidans* possesses genes involved in assimilatory sulfate reduction (*cysIHDNCG*), a trait that is not found in most other GSB. Given the irregular distribution of certain enzymes (such as FccAB, AprAB, Sat, QmoABC) among GSB strains, it appears that different enzymes may produce the same sulfur oxidation phenotype in different strains. Finally, even though the GSB are closely related, sequence analyses show that the sulfur metabolism gene content in these bacteria is substantially influenced by gene duplication and elimination and by lateral gene transfer both within the GSB phylum and with prokaryotes from other phyla.

## I. Introduction

Inorganic sulfur metabolism in prokaryotic organisms is a complicated topic due to the complex chemistry of sulfur and the multitude of enzymes that have evolved to catalyze this chemistry. Nevertheless, the ability to use inorganic sulfur compounds as electron sources for growth is widespread among very different prokaryotes of both archaeal and bacterial affiliation. Phototrophic sulfur bacteria characteristically oxidize reduced inorganic sulfur compounds for photoautotrophic growth under anaerobic conditions. These bacteria are traditionally divided into the green sulfur bacteria (GSB) and the purple sulfur bacteria (PSB). The ecology of GSB and PSB is to some extent similar (van Gemerden and Mas, 1995; Overmann, 2007) and their oxidative sulfur metabolism probably shares many characteristics as well (Brune, 1989, 1995). However, other aspects of their physiology, evolution, and taxonomy are rather different. These differences are reflected in current taxonomic assignments of these two groups of *Bacteria*: The GSB comprise the phylum *Chlorobi,* whereas all PSB are members of the physiologically highly diverse phylum *Proteobacteria* (Boone and Castenholz, 2001).

GSB are obligately anaerobic and obligately photoautotrophic, and they form a phylogenetically and physiologically distinct group (Overmann, 2000; Garrity and Holt, 2001; Imhoff, 2007). They are commonly found in anoxic and sulfide-rich freshwater and estuarine environments, either in the water column, in sediments, or within microbial mats. They have also recently been found in the anoxic zone 100 m below the surface of the Black Sea (Overmann et al., 1992; Manske et al., 2005), on deep-sea hydrothermal vents in the Pacific Ocean (Beatty et al., 2005), and in the microbial mats of Octopus and Mushroom Springs in Yellowstone National Park (Ward et al., 1998). All GSB characterized to date have unique light-harvesting organelles known as chlorosomes, which allow highly efficient

*Abbreviations*: *Alc*. – *Allochromatium*; APS – adenosine-5′-phosphosulfate (also called adenylylsulfate); *Cba*. – *Chlorobaculum*; *Chl*. – *Chlorobium*; GSB – green sulfur bacteria; PAPS – 3′-phosphoadenosine-5′-phosphosulfate (also called 3′-phosphoadenylylsulfate); *Pld*. – *Pelodictyon*; PSB – purple sulfur bacteria; PSR – polysulfide reductase; PSRLC – polysulfide reductase-like complex; *Ptc*. – *Prosthecochloris*; SQR – sulfide:quinone reductase; SQRLP – sulfide:quinone reductase-like protein

*Table 1*. Strains of green sulfur bacteria selected for genome sequencing.

| Strain designations[a] | Names used[b] | Phenotype[c] | Genome sequence status[d] |
|---|---|---|---|
| ATCC 35110$^T$ | *Chloroherpeton thalassium* | BChl *c* | 3.25 Mbp (1 chromosome; 3 gaps) |
| TLS, ATCC 49652$^T$ | *Chlorobaculum tepidum*, (*Chlorobium tepidum*) | BChl *c*, Tio$^+$ | 2,154,946 bp (1 chromosome) |
| BS1 | *Prosthecochloris* sp., (*Chlorobium phaeobacteroides*) | BChl *e* | 2,736,402 bp ( 1 chromosome) |
| CaD3 | *Chlorobium chlorochromatii*, (*"Chlorochromatium aggregatum"* epibiont) | BChl *c*, Tio$^-$ | 2,572,079 bp (1 chromosome) |
| DSMZ 245$^T$, 6330 | *Chlorobium limicola* | BChl *c*, Tio$^-$ | 2,763,183 bp (1 chromosome) |
| DSMZ 263$^T$, NCIMB 8327 | *Chlorobaculum parvum*, (*Chlorobium vibrioforme* subsp. *thiosulfatophilum*, *Chlorobium thiosulfatophilum*) | BChl *d*, Tio$^+$ | 2,289,236 bp (1 chromosome) |
| DSMZ 265, 1930 | *Chlorobium phaeovibrioides*, (*Chlorobium vibrioforme* subsp. *thiosulfatophilum*) | BChl *c* + *d*, Tio$^+$ | 1,966,858 bp (1 chromosome) |
| DSMZ 266$^T$, 2430 | *Chlorobium phaeobacteroides* | BChl *e*, Tio$^-$ | 3,133,902 bp (1 chromosome) |
| DSMZ 271$^T$, SK-413 | *Prosthecochloris aestuarii* | BChl *c*, Tio$^-$ | 2,512,923 bp (1 chromosome) |
| DSMZ 273$^T$, 2530 | *Chlorobium luteolum*, (*Pelodictyon luteolum*) | BChl *c*, Tio$^-$ | 2,364,842 bp (1 chromosome) |
| DSMZ 5477$^T$, BU-1 | *Chlorobium clathratiforme*, (*Pelodictyon phaeoclathratiforme*) | BChl *e*, Tio$^+$ | 2,018,240 bp ( 1 chromosome) |
| DSMZ 13031$^T$ | *Chlorobium ferrooxidans* | BChl *c*, Tio$^-$ | 2.6 Mbp (draft available; 5 gaps) |

[a] Superscript "T" denotes type strain.
[b] Names are according to Imhoff (2003) except those in parenthesis, which represents alternative names.
[c] Tio$^+$ indicates that thiosulfate is utilized for growth.
[d] Status as of November 2007.

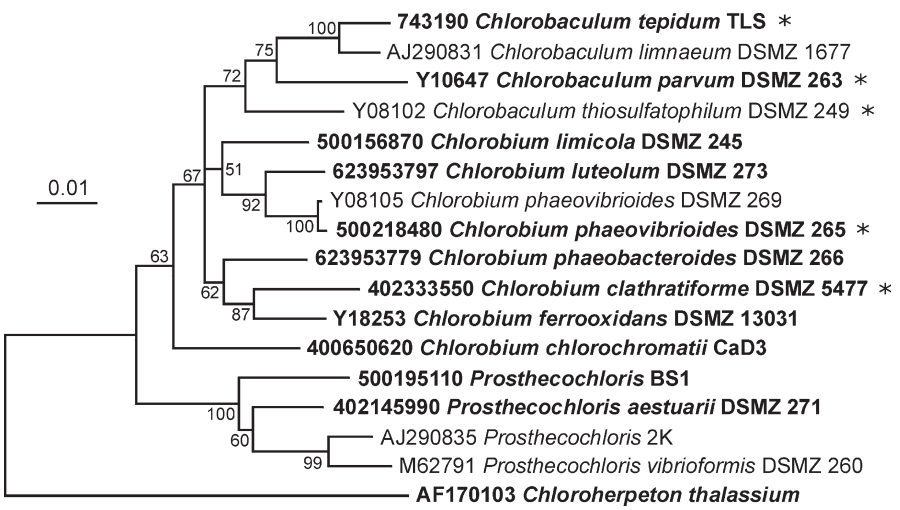


*Fig. 1*. Unrooted neighbor-joining phylogenetic tree of the 16S rRNA gene of selected GSB strains. Strains that have been selected for genome sequencing are shown in bold. Strains that have been shown to grow on thiosulfate are indicated with an asterisk. Sequence accession numbers are either from the JGI data base (digits only) or from GenBank. The tree is based on 1119 nucleotide positions and was made with MEGA version 3.1 (Kumar et al., 2004). Bootstrap values in percentage are shown for 1000 replications. Except for the position of *Chl. chlorochromatii* CaD3, whose position is not resolved, minimum evolution and maximum parsimony analyses also support the topology of this tree.

capture of light energy; therefore, these organisms can grow at remarkably low light intensities. All characterized strains use the reductive (also called reverse) tricarboxylic acid cycle for $CO_2$ fixation. Most strains use electrons derived from oxidation of sulfide, thiosulfate, elemental sulfur, and $H_2$, but a few characterized strains can also oxidize $Fe^{2+}$.

In an effort to learn more about the physiology and evolution of this unique group, and especially about their photosynthesis and carbon and sulfur metabolism, 12 strains of GSB have been selected for genome sequencing (Table 1). Figure 1 shows a 16S rRNA phylogenetic tree of these and other GSB strains. The genome of one of the best characterized strains, *Chlorobaculum tepidum* TLS (previously known as *Chlorobium tepidum* TLS), was sequenced and annotated in 2002 by The Institute for Genomic Research (TIGR; Eisen et al., 2002). Eleven other strains are currently at various stages of genome sequencing and annotation at the Joint Genome Institute (United States Department of Energy) and in the laboratories of Donald A. Bryant (The Pennsylvania State University, United States) and Jörg Overmann (Ludwig-Maximilians-Universität, Germany) (Table 1). The genome sequences can be accessed and analyzed on the websites of the Integrated Microbial Genomes resource (http://img.jgi.doe.gov) and the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). Recent reviews on information derived from genome sequence data of GSB are available (Frigaard et al., 2003, 2006; Frigaard and Bryant, 2004). Here, we present an overview of the content and phylogeny of known or putative enzymes involved in inorganic sulfur metabolism in the 10 strains of the GSB for which sufficient genome sequence information is currently available (all strains listed in Table 1, except *Chlorobaculum parvum* DSMZ 263 and *Chloroherpeton thalassium* ATCC 35110). Sulfur metabolism in GSB is also discussed in the chapter by Hanson (2008). Current knowledge on the oxidation of inorganic sulfur compounds in PSB is reviewed in the chapter by Dahl (2008). At present, genome sequence information is publicly available only for one PSB: the halophilic *Halorhodospira halophila* SL1 (DSMZ 244) of the *Ectothiorhodospiraceae* family (http://www.ncbi.nlm.nih.gov).

## II. Sulfur Compounds Oxidized for Growth

Almost all GSB are capable of oxidizing sulfide and elemental sulfur to sulfate. GSB have a high affinity for sulfide, and sulfide is usually the preferred substrate even if other sulfur substrates are available (Brune, 1989, 1995). Initially, sulfide is typically incompletely oxidized to elemental sulfur, which is deposited extracellularly as sulfur globules. These sulfur globules are oxidized completely to sulfate when the sulfide has been consumed. Some of the sulfur compounds utilized by the strains for which genome sequence data are available are shown in Table 2.

Several strains of GSB are also capable of growing on thiosulfate ($S_2O_3^{2-}$) (see overview in Imhoff, 2003). Some of these strains can grow on thiosulfate in the absence of any other reduced sulfur compound (N.-U. Frigaard, unpublished data). Two thiosulfate-utilizing strains of GSB have been reported to utilize tetrathionate ($S_4O_6^{2-}$) (Brune et al., 1989). No GSB has been reported to utilize sulfite ($SO_3^{2-}$).

## III. Sulfur Compound Oxidation Enzymes

Many enzymes potentially involved in sulfur metabolism can readily be identified in the genome sequences by sequence homology with known enzymes. Table 2 shows a detailed survey of genes known to be involved or potentially involved in the oxidative sulfur metabolism in the genome-sequenced strains of GSB. Specific enzymes are discussed below. An overview of the metabolic network formed by these enzymes is shown in Fig. 2.

### *A. Sulfide:Quinone Reductase*

Sulfide:quinone reductase (SQR; EC 1.8.5.-) catalyzes oxidation of sulfide with an isoprenoid quinone as the electron acceptor. This enzyme occurs in both chemotrophic and phototrophic prokaryotes as well as in some mitochondria (Griesbeck et al., 2000; Theissen et al., 2003). Membrane-bound SQR activity has been biochemically demonstrated in GSB and presumably feeds electrons into the photosynthetic electron transfer chain

*Table 2*. Phenotypes and genotypes of green sulfur bacteria for which genome sequence data is available.

| | Electron donor[a] | | | Genotype[b] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain | $S^{2-}$ | $S^0$ | $S_2O_3^{2-}$ | *sqr* | *dsr* | *fcc* | *soyYZ* | *sox* | *apr* | *sat* | *qmo* | *hdr-hyd* | PSRLC1 | PSRLC2 | PSRLC3 | *hup* | *cys* |
| *Chlorobaculum tepidum* TLS | + | + | + | + | + | + | − | + | + | + | + | + | + | − | − | − | − |
| *Chlorobium chlorochromatii* CaD3 | + | − | − | + | + | + | − | + | + | + | + | + | + | + | + | − | − |
| *Chlorobium clathratiforme* DSMZ 5477 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | − |
| *Chlorobium ferrooxidans* DSMZ 13031 | − | − | − | + | − | − | − | − | − | − | − | + | − | − | − | + | + |
| *Chlorobium limicola* DSMZ 245 | + | + | − | + | + | + | + | − | − | − | − | + | − | + | + | + | − |
| *Chlorobium luteolum* DSMZ 273 | + | + | − | + | + | − | − | − | − | − | − | + | + | − | + | + | + |
| *Chlorobium phaeobacteroides* DSMZ 266 | + | + | − | + | + | + | + | − | − | − | − | + | − | + | + | + | − |
| *Chlorobium phaeovibrioides* DSMZ 265 | + | + | + | + | + | + | − | + | − | − | − | + | + | − | + | − | − |
| *Prosthecochloris* sp. BS1 | + | | | + | + | + | − | − | + | + | + | − | + | + | − | + | − |
| *Prosthecochloris aestuarii* DSMZ 271 | + | + | − | + | + | + | + | − | − | − | − | − | − | + | + | + | − |

[a] Garrity and Holt 2001, Heising et al. 1999, Vogl et al. 2006.
[b] The following abbreviations designates more than one gene: apr: *aprBA, cys: cysIHDNCG-cysPTWA, dsr: dsrABCEFHLNMKJOPT, fcc: fccAB, hdr-hyd: hdrDA-orf1247-orf1248-hydB2G2, hup: hupSLCD, sox: soxJXYZAKBW, qmo: qmoABC.*

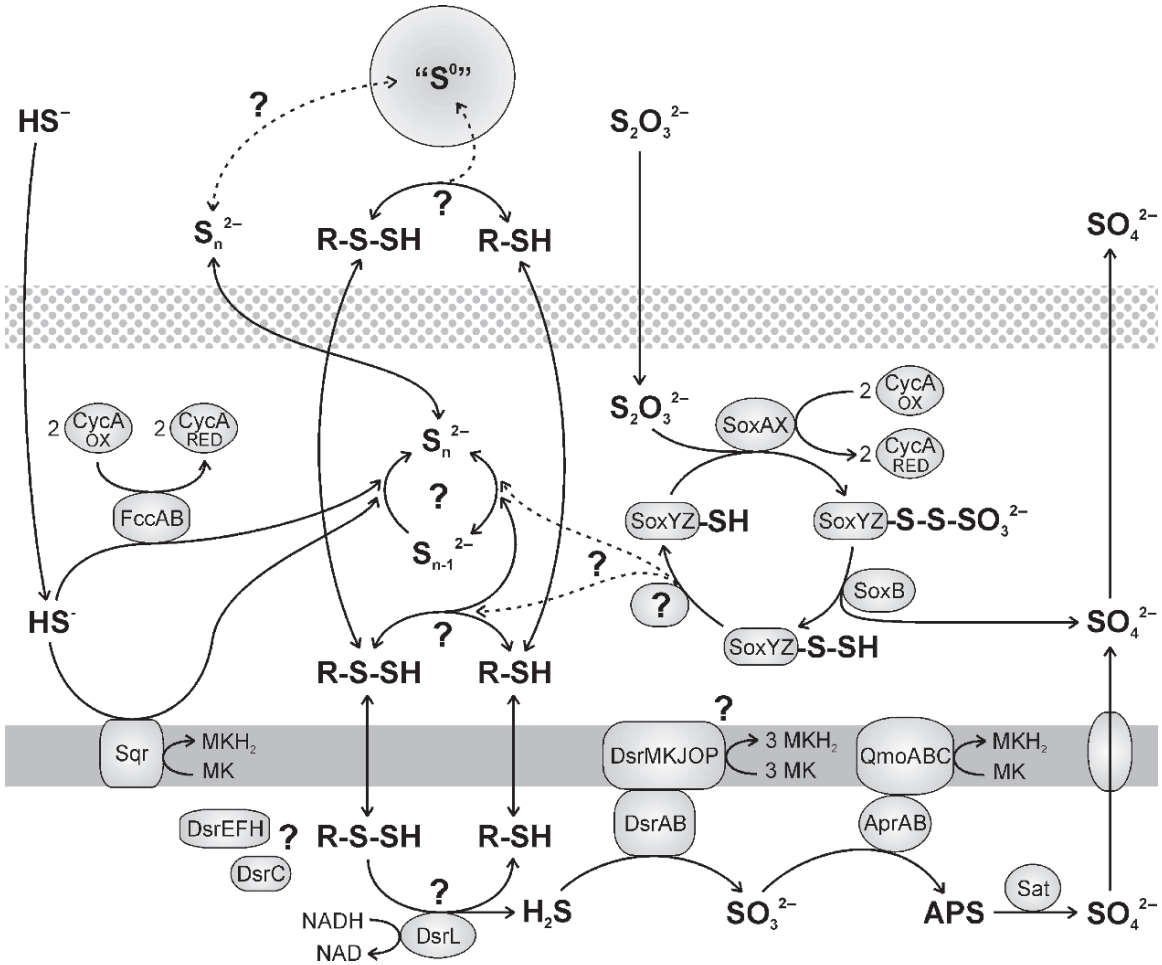


*Fig. 2*. Overview of known or hypothesized pathways in the oxidation of inorganic sulfur compounds in GSB. Not all strains have all pathways shown here. This figure is derived from information in Eisen et al., 2002 and Dahl, 2008. (See text for details.)

via a quinol-oxidizing Rieske iron-sulfur protein/cytochrome *b* complex (Shahak et al., 1992). The genome sequences of all GSB strains encode one (CT0117 in *Cba. tepidum* TLS) or two homologs of the biochemically characterized SQRs from *Rhodobacter capsulatus* (CAA66112) and *Oscillatoria limnetica* (AAF72962) (Fig. 3). This includes *Chl. ferrooxidans* (ZP_01385816), which cannot grow on sulfide as the sole electron donor (Heising et al., 1999). This organism may benefit from SQR activity as a supplement to its energy metabolism. It could also use SQR as a protective mechanism to remove sulfide, which prevents growth when present in high concentrations. The SQR homologs of GSB are flavoproteins with predicted masses of about 53 kDa, and each contains all three conserved cysteine residues that are essential for sulfide oxidation in *Rhodobacter capsulatus* SQR (Griesbeck et al., 2002).

Some GSB additionally contain distantly related homologs of SQR with no assigned or obvious function (here denoted SQRLP1 and SQRLP2 for SQR-like proteins type 1 and 2) (Fig. 3). SQRLP1 is present in *Cba. tepidum* TLS (CT1087) and in three other GSB. Among the GSB, SQRLP2 is only present in *Cba. tepidum* TLS (CT0876). Interestingly, SQRLP2 clusters in phylogenetic analyses with proteins of unknown function from various archaea. In addition, SQRLP2 from *Cba. tepidum* TLS shares 54% amino acid sequence identity with a protein from *Sulfitobacter* strain NAS14.1, which is a marine

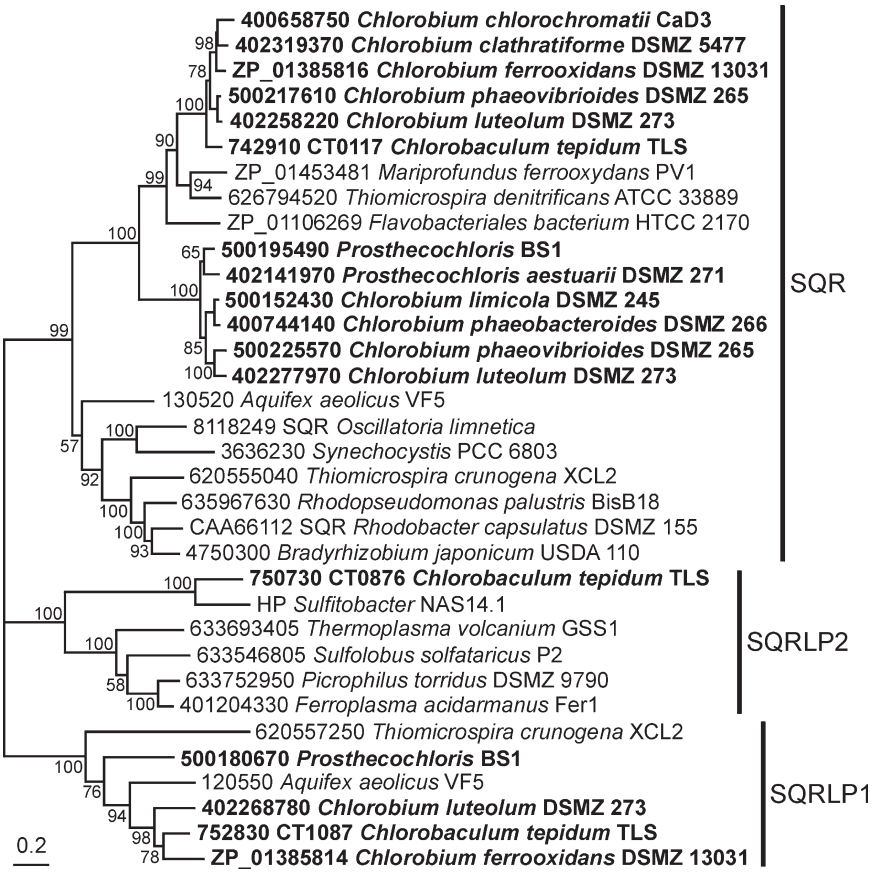


*Fig. 3*. Unrooted neighbor-joining phylogenetic tree of sulfide: quinone reductase (SQR) and two types of SQR-like proteins (SQRLP1 and SQRLP2) in selected organisms. Strains of GSB are shown in bold. The tree was made with MEGA version 3.1 (Kumar et al., 2004) and shows bootstrap values for 100 replications.

*Roseobacter*-like strain that grows aerobically on dimethylsulfoniopropionate (https://research.venterinstitute.org/moore/). Otherwise, SQRLP2 has few homologs in the databases.

### *B. Flavocytochrome* c

Flavocytochrome *c* is usually a soluble, periplasmic enzyme consisting of a large sulfide-binding FccB flavoprotein subunit and a small FccA cytochrome *c* subunit (Brune, 1995). Although this protein efficiently oxidizes sulfide and reduces cytochrome *c* in vitro, the exact function and significance of this protein in vivo is still not clear. Although many sulfide-utilizing organisms produce flavocytochrome *c*, some sulfide-utilizing GSB and PSB do not, which clearly demonstrates that flavocytochrome *c* is not essential for sulfide oxidation (Brune, 1995). For example, the pattern of sulfide oxidation and the concomitant formation of elemental sulfur that is subsequently oxidized to sulfate upon sulfide depletion, is similar in *Chl. luteolum* DSMZ 273, which does not contain flavocytochrome *c*, and in *Chl. limicola* DSMZ 245, which does contain flavocytochrome *c* (Steinmetz and Fischer, 1982). In addition, a mutant of the PSB *Allochromatium vinosum* DSMZ 180, in which flavocytochrome *c* has been eliminated genetically, exhibits sulfide and thiosulfate oxidation rates similar to the wild type (Reinartz et al., 1998). If indeed the FccAB flavocytochrome *c* oxidizes sulfide in vivo, both GSB and PSB apparently have alternative sulfide-oxidizing enzyme systems, possibly sulfide:quinone reductase (section III.A) and the Dsr system (section III.C), that may be quantitatively more important.

However, it is also possible that flavocytochrome *c* is advantageous under certain growth conditions and that such conditions have not yet been identified in these bacteria.

An *fccAB*-encoded flavocytochrome *c* is found in all GSB strains for which genome sequence data is available, except *Chl. ferrooxidans* DSMZ 13031 and *Chl. luteolum* DSMZ 273. The GSB flavocytochrome *c* consists of a 10-kDa FccA cytochrome $c_{553}$ subunit (CT2080 in *Cba. tepidum* TLS) that binds a single heme and an approximately 47-kDa sulfide-binding FccB flavoprotein subunit (CT2081 in *Cba. tepidum* TLS). The FccB subunit has high sequence similarity (approximately 50% amino acid sequence identity) to a flavoprotein (SoxJ) encoded in the *sox* gene cluster (section III. G.1). FccAB is constitutively expressed in *Cba. thiosulfatiphilum* (formerly *Chl.limicola* subsp. *thiosulfatophilum*) DSMZ 249 (Verté et al., 2002). In this strain, FccAB was reported to be membrane-bound, possibly due to an unusual signal peptide in the FccA subunit that is not cleaved but supposedly anchors the protein in the cytoplasmic membrane. The predicted signal peptide in FccA from GSB is followed by a highly variable, 15- to 25-residue sequence, which is rich in alanine and proline and which is suggested to act as a flexible arm (Verté et al., 2002).

### C. Dissimilatory Sulfite Reductase

The well-studied PSB, *Alc. vinosum,* contains a gene cluster with high sequence similarity to the dissimilatory sulfite reductase *dsr* gene cluster of sulfate-reducing bacteria (Dahl et al., 2005). The *dsr* gene cluster in *Alc. vinosum*, *dsrABEFHCMKLJOPNRS*, is essential for the oxidation of intracellular sulfur globules, and thus it is assumed that the Dsr enzyme system in this organism functions in the oxidative direction to produce sulfite (Pott and Dahl, 1998; Dahl et al., 2005; Sander et al., 2006). All GSB, except *Chl. ferrooxidans*, contain the *dsrABCEFHLNMKJOPT* genes. Thus, despite the absence of *dsrRS* and the presence of *dsrT* in the Dsr system in GSB, this system most likely functions very similarly to the Dsr enzyme system in PSB. The absence of *dsr* genes in *Chl. ferrooxidans* is consistent with the observation that this bacterium is incapable of growth on elemental sulfur and sulfide.

In *Cba. tepidum* TLS the *dsr* genes are split into two clusters, and three functional *dsr* genes are duplicated (*dsrA*, *dsrC*, and *dsrL*). This may be due to a frameshift mutation in the *dsrB* gene in a recent ancestor of the TLS strain that rendered the gene nonfunctional. This could have selected for a duplication, rearrangement and subsequent frameshift mutation of a small segment of the genome, which restored a functional *dsrB* gene but also resulted in a duplication of the *dsrCABL* gene cluster. The two regions that contain a *dsrCABL* cluster in *Cba. tepidum* TLS are 99.4% identical at the nucleotide level. From the currently available data it appears that the *dsr* genes only occur as a single cluster in all other genome-sequenced GSB.

Based upon phylogenetic analyses, the cytoplasmic DsrAB sulfite reductase and other cytoplasmic Dsr proteins in GSB are related to the Dsr proteins from other sulfide-oxidizing prokaryotes (Sander et al., 2006). However, the subunits of the membrane-bound DsrMKJOP complex are related to the DsrMKJOP proteins from sulfate-reducing prokaryotes. In addition, the DsrT protein (unknown function) is only found in GSB and sulfate-reducing prokaryotes and not in other sulfide-oxidizers. This suggests an intriguing chimeric nature of the Dsr system in GSB, possibly generated by lateral gene transfer of *dsrTMKJOP* from a sulfate-reducing prokaryote to a common ancestor of GSB. An interesting possibility is that it might have been the acquisition of the ability to oxidize sulfur to sulfite that led to the relatively recent, explosive radiation of the GSB.

### D. Sulfite Oxidation

Although GSB cannot grow on sulfite as sole sulfur source and electron donor, sulfite appears to be the product of the Dsr enzyme system (section III.C, Fig. 2). Sulfite can be oxidized by adenosine-5′-phosphosulfate (APS) reductase (also called adenylylsulfate reductase, EC 1.8.99.2) in a reaction that consumes sulfite and AMP and generates APS and reducing equivalents. Two non-homologous types of such enzymes are known: the AprAB type that functions in dissimilatory sulfur metabolism and the CysH type that functions in assimilatory sulfur metabolism. An Apr-type APS reductase is found in the genomes of four GSB (Table 2). In *Cba. tepidum* TLS the genes are *aprA/CT0865* and *aprB/CT0864*. A CysH-type APS reductase is

found in *Chl. luteolum* DSMZ 273 and *Chl. ferrooxidans* DSMZ 13031, in which this enzyme probably functions in assimilatory sulfate reduction (see section VI). No APS reductase has been identified in the genomes of other GSB. Despite the absence of a recognizable APS reductase in its genome sequence, an APS reductase activity has been purified from *Chl. limicola* DSMZ 245 and biochemically characterized although not sequenced (Kirchhoff and Trüper, 1974). This APS reductase from *Chl. limicola* DSMZ 245 was reported to have a molecular mass of about 200 kDa and to contain one flavin per molecule and non-heme iron, but it contained no heme. Homologs of the molybdopterin-binding SorAB sulfite:cytochrome *c* oxidoreductase (EC 1.8.2.1) from *Starkeya novella* (formerly *Thiobacillus novellus*) (Kappler et al., 2000) are not found in any GSB genome sequence. An alternative putative molybdopterin-binding enzyme that may function in sulfite oxidation in GSB is described in section IV.B.

### *E. Release of Sulfate from APS*

AMP-dependent oxidation of sulfite by APS reductase produces APS (section III.D). APS can be hydrolyzed to AMP and sulfate by adenylylsulfatase (EC 3.6.2.1) but there is no evidence for this enzyme in GSB. Alternatively, the energy of the phosphosulfate anhydride bond in APS can be conserved by the action of sulfate adenylyltransferase (also called ATP-sulfurylase; EC 2.7.7.4) encoded by the *sat* gene. ATP-sulfurylase generates ATP and sulfate from APS and pyrophosphate. ADP-sulfurylase (EC 2.7.7.5) generates ADP and sulfate from APS and phosphate (this enzyme is also called adenylylsulfate:phosphate adenylyltransferase, APAT). Some GSB strains have been reported biochemically to contain either ATP-sulfurylase activity (strains DSMZ 249 and DSMZ 257) or ADP-sulfurylase activity (strains DSMZ 263 and NCIMB 8346) but not both activities (Khanna and Nicholas, 1983; Bias and Trüper, 1987); however, the genome of none of these strains has been sequenced. Four GSB genomes encode highly similar, *sat*-encoded ATP-sulfurylases (Table 2; CT0862 in *Cba. tepidum* TLS).

Another type of ATP-sulfurylase, which is not homologous with the Sat enzyme, is the heterodimeric CysDN known from assimilatory sulfate reduction in *Alc. vinosum*, *Escherichia coli*, and other prokaryotes (Kredich, 1996; Neumann et al., 2000). Genes encoding a CysDN-like complex are found in *Chl. ferrooxidans* and *Chl. luteolum* DSMZ 273 as part of an assimilatory sulfate reduction gene cluster (section VI). *Chl. phaeovibrioides* DSMZ 265 and *Ptc. aestuarii* DSMZ 271 also contain *cysC* and *cysN* homologs. But in these two latter organisms the genes occur in a cluster that appears to be involved in another aspect of sulfur metabolism. This cluster also contains a *cysQ* homolog that may encode a phosphatase acting on APS or PAPS (Neuwald et al., 1992) and a homolog of the ArsB/NhaD superfamily of permeases that translocates $Na^+$ and various anions such as sulfate across the cytoplasmic membrane (500231320 and 500231330, respectively, in *Chl. phaeovibrioides* DSMZ 265). It is therefore possible that *Chl. phaeovibrioides* DSMZ 265 and *Ptc. aestuarii* DSMZ 271 possess a system that processes APS or sulfite (or both) differently than in other GSB and that actively excretes sulfate. No homologs of *sat*, *cysN*, or *cysD* have been identified in *Chl. limicola* DSMZ 245 or *Chl. phaeobacteroides* DSMZ 266 and it is not clear how these strains convert APS to sulfate, if they do at all.

### *F. Qmo Complex*

*Cba. tepidum* TLS and three other GSB strains (Table 2) contain a membrane-bound electron-transfer complex (QmoA/CT0866-QmoB/CT0867-QmoC/CT0868) that shares homology with subunits of heterodisulfide reductases. The Qmo complex from GSB has the same subunit structure and the same putative cofactor-binding sites as the Qmo complex in the sulfate-reducing organisms, *Desulfovibrio desulfuricans* and *Archaeoglobus fulgidus* (Pires et al., 2003). It appears that in all these organisms QmoA and QmoB are cytoplasmic, nucleotide- and iron–sulfur-cluster-binding subunits and QmoC is a membrane-bound, heme-*b*-binding subunit that exchanges electrons with the isoprenoid quinone pool. The Qmo complex from *Desulfovibrio desulfuricans* was biochemically characterized and shown to have quinol-oxidizing activity (Pires et al., 2003). In all four cases in which the genes encoding a Qmo complex (*qmoABC*), an ATP-

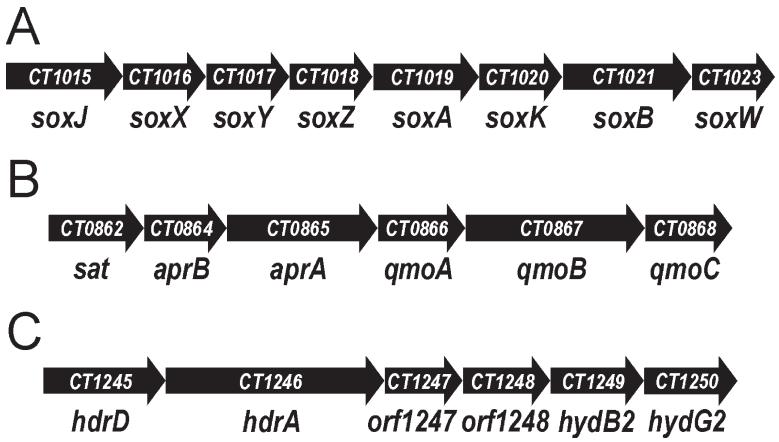


*Fig. 4*. Gene clusters in *Cba. tepidum* TLS of (A) *sox* genes, (B) *sat-apr-qmo* genes, and (C) *hdr-hyd* genes. These clusters are conserved in all GSB strains in which the genes occur.

sulfurylase (*sat*), and an APS reductase (*aprAB*) occur in GSB, these genes are clustered in one apparent *sat-aprBA-qmoABC* operon (Table 2 and Fig. 4B). A similar *aprBA-qmoABC* operon with high sequence similarity occurs in *Desulfovibrio desulfuricans* and other *Desulfovibrio*-like strains. It therefore seems likely that the four GSB that have this operon may have obtained it from a *Desulfovibrio*-like organism by lateral gene transfer. The enzymes encoded by the *sat-aprBA-qmoABC* operon in principle allow oxidation of sulfite to sulfate via an APS intermediate with concomitant reduction of membrane-bound quinones (Fig. 2). How this reaction occurs in GSB that do not have the *sat-aprBA-qmoABC* genes is not clear (see Section IV.B for a possible alternative sulfite oxidation system).

## G. Thiosulfate Oxidation

### 1. Sox System

In the chemolithoautotrophic α-proteobacterium *Paracoccus pantotrophus* the *sox* gene cluster comprises 15 genes that encode proteins involved in the oxidation of thiosulfate and possibly other sulfur compounds (Friedrich et al., 2001, 2005). The Sox proteins are transported to the periplasm, either by encoding a signal peptide recognized by the Sec system or the Tat system, or by forming a complex with another Sox protein that encodes a signal peptide. The products of seven *sox* genes, *soxXYZABCD*, are induced by thiosulfate and are sufficient to reconstitute a thiosulfate-oxidizing enzyme system in vitro. Friedrich et al. (2001) proposed the following model for thiosulfate oxidation: The sulfane atom of thiosulfate is bound to the SoxYZ complex (SoxYZ-SH) by an oxidation reaction, catalyzed by the SoxAX *c*-type cytochrome, which results in a thiocysteine-*S*-sulfate residue (SoxYZ-S-S-$SO_3^{2-}$). A conserved cysteine residue in the SoxY subunit constitutes the substrate-carrying site. Sulfate is liberated by hydrolysis catalyzed by SoxB to yield a persulfide intermediate (SoxYZ-S-$S^-$), which subsequently is oxidized by another *c*-type cytochrome, SoxCD, to form a cysteine-S-sulfate residue (SoxYZ-S-$SO_3^{2-}$). Hydrolysis by SoxB releases sulfate and regenerates the SoxYZ complex (SoxYZ-SH).

The cluster of *sox* genes in *Cba. tepidum* TLS, *CT1015-soxXYZA-CT1020-soxBW* (Fig. 4A), is conserved in the genomes of three other strains (Table 2). Because of the organizational conservation and the congruent phylogeny of the eight genes in this cluster (see section VII), the genes *CT1015* and *CT1020* are likely involved in the Sox system. Thus, these two genes are now denoted as *soxJ* and *soxK*, respectively. The *soxJXYZAKBW* cluster is present in all three thiosulfate-utilizing strains (TLS, DSMZ 265, DSMZ 5477) and one strain (CaD3) that has not been reported to grow on thiosulfate. All SoxY proteins in GSB have the conserved C-terminal

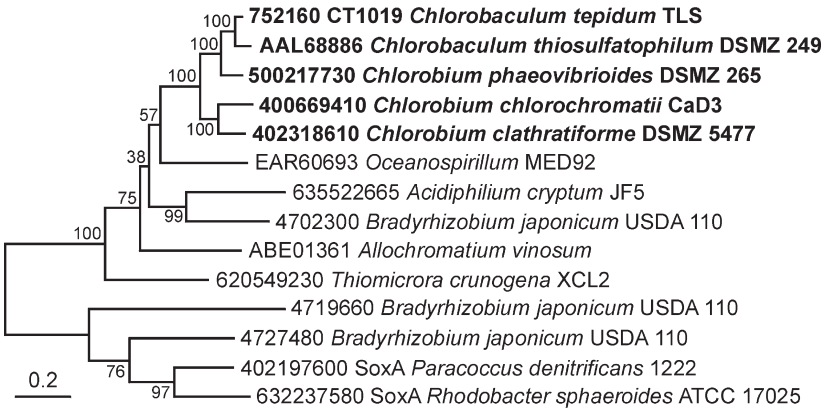


*Fig. 5*. Unrooted neighbor-joining phylogenetic tree of SoxA proteins. Strains of GSB are shown in bold. The tree was made with MEGA version 3.1 (Kumar et al., 2004) and shows bootstrap values for 100 replications.

motif GGCGG-COOH with the substrate-binding cysteine residue. (The genes encoding the SoxYZ complex have been duplicated in some GSB; see section III.H.) The gene *soxJ* encodes a putative FAD-containing dehydrogenase related to the sulfide-binding flavoprotein subunit of flavocytochrome *c*. The gene *soxK* encodes a hypothetical 11-kDa protein with a signal peptide and with a homolog encoded in the *sox* cluster of the purple sulfur bacterium *Alc. vinosum* (ABE01362), but has no identified homologs in other organisms. SoxA from bacteria such as *P. pantotrophus* and *Rhodovulum sulfidophilum* binds heme groups in two conserved CXXCH motifs (Appia-Ayme et al., 2001; Bamford et al., 2002). The C-terminal heme presumably participates in oxidation of the thiosulfate-SoxYZ complex; the N-terminal heme is presumably too far from the C-terminal heme to allow electron transfer between the two heme groups, and its function is not known. SoxA in GSB and some other bacteria such as *Alc. vinosum* and *Starkeya novella* only has the C-terminal heme-binding motif. This difference is reflected in a phylogenetic sequence analysis, in which SoxA sequences from GSB and *Alc. vinosum* group separately from the SoxA sequences of *P. denitrificans* and *Rhodobacter sphaeroides* that have two heme-binding motifs (Fig. 5).

The *soxCD* genes, which are essential components of the Sox system in *P. pantotrophus*, do not occur in the genome sequences of GSB. The *soxCD* genes have also not been found in the purple sulfur bacterium *Alc. vinosum* (Hensen et al., 2006). This observation suggests (1) that the persulfide-form of the SoxYZ carrier protein (SoxYZ-S-S$^-$) is transformed back to the unmodified form (SoxYZ-SH) differently in GSB and in *P. pantotrophus,* and (2) that the sulfane moiety from the thiosulfate molecules that become attached to the SoxYZ carrier protein are not completely oxidized to sulfate in the GSB Sox system as they are in the *P. pantotrophus* Sox system. This is consistent with experimental evidence in *Alc. vinosum,* which shows that the sulfane moiety from thiosulfate is found as elemental sulfur when sulfur globules are formed by oxidation of thiosulfate (Smith and Lascelles, 1966; Trüper and Pfennig, 1966). In this process, a net electron gain in the GSB Sox system is only accomplished by the SoxAX-dependent oxidation (Fig. 2). The SoxCD-independent reaction in GSB that regenerates the SoxYZ complex may involve the SoxJ and SoxK proteins due to the conservation of their genes in the GSB *sox* gene cluster (Fig. 4A).

### 2. Rhodaneses

Rhodaneses (thiosulfate sulfurtransferases, EC 2.8.1.1) are enzymes that catalyze the transfer of the sulfane sulfur atom of thiosulfate to cyanide ($CN^-$) to generate thiocyanate ($SCN^-$) and $SO_3^{2-}$. However, this is often not the physiological role of the enzymes. Rhodaneses are common in many organisms, including phototrophic sulfur bacteria, but their roles in lithotrophic sulfur metabolism are not clear (Brune, 1995). Two rhodaneses

with unknown function have been purified from the thiosulfate-utilizing *Cba. parvum* DSMZ 263 of which the most abundant and active rhodanese was a 39-kDa basic protein with an isoelectric point of 9.2 (Steinmetz and Fischer, 1985).

All GSB, other than *Chl. ferrooxidans*, contain a 17-kDa rhodanese (CT0843 in *Cba. tepidum* TLS) as part of the *dsr* gene cluster (section III.C). Other putative rhodaneses are found scattered among the genome sequences of GSB in a manner that does not obviously correspond with their ability to use thiosulfate. A putative periplasmic 22-kDa rhodanese with an isoelectric point of 8.8 is found in the thiosulfate-utilizing *Chl. clathratiforme* DSMZ 5477 (ZP_00590525). A homolog of this protein is found in *Chl. chlorochromatii* CaD3 (ABB28218), which has not been reported to use thiosulfate, but homologs are not found in the genome sequences of other GSB. *Chl. limicola* DSMZ 245 and *Prosthecochloris* sp. BS1 contain an acidic, putatively cytoplasmic, ~ 50-kDa rhodanese (ZP_00512484 and ZP_00530387, respectively), which is homologous with a putative rhodanese found in *Salinibacter ruber* (53% sequence identity) and several strains of *Escherichia coli* (37% sequence identity).

#### 3. Plasmid-Encoded Thiosulfate Oxidation?

A 15-kb plasmid, named pCL1, has been isolated from the thiosulfate-utilizing *Cba. thiosulfatiphilum* DSMZ 249 (Méndez-Alvarez et al., 1994). When this plasmid was transferred to *Chl. limicola* DSMZ 245, which cannot grow on thiosulfate, the resulting transformants were reported to utilize thiosulfate as the sole electron donor for growth. The plasmid has been sequenced (NC_002095), but surprisingly it does not encode genes known to be involved in thiosulfate utilization (C. Jakobs et al., unpublished data). No putative enzymes involved in sulfur chemistry are encoded by the plasmid, but it is possible that the plasmid somehow allows cellular transport of certain sulfur compounds. The plasmid contains a cluster of seven genes (similar to *cpaBCEF-tadBC-pilD*), which are homologs of genes involved in a type II secretion system that functions in pilus formation in many bacteria. The other genes on the pCL1 plasmid are apparently involved in plasmid maintenance. Three of the seven genes in the *cpaBCEF-tadBC-pilD*-like cluster on the plasmid contain frame-shift mutations that probably cause non-functional proteins. (Alternatively, these mutations could be due to sequencing errors.) A highly similar cluster, but with the reading frame of all genes intact, is found in the genome sequences of all three GSB that can grow on thiosulfate (strains TLS, DSMZ 265, DSMZ 5477), as well as in one strain that cannot grow on thiosulfate (DSMZ 273), but not in other strains. Since *Chl. chlorochromatii* CaD3 is the only strain that has the *Chlorobium*-type *sox* cluster (Fig. 4A) but cannot grow on thiosulfate (Table 2), one can speculate that the inability of this strain to grow on thiosulfate is due to the absence of the *cpaBCEF-tadBC-pilD*-like genes. The GSB-type *sox* cluster is also present in *Cba. thiosulfatiphilum* DSMZ 249 (AY074395) from which plasmid pCL1 was isolated, and presumably is located on the chromosome (Verté et al., 2002). It is not clear from the genome sequence how *Chl. limicola* DSMZ 245 can grow on thiosulfate after receiving the pCL1 plasmid (Méndez-Alvarez et al., 1994). Strain DSMZ 245 does not have *sox* genes and has no other obvious candidate for a thiosulfate-metabolizing enzyme, other than a putative cytoplasmic rhodanese (see above) and the putative novel enzyme system FccAB-SoyYZ (see section III.H). Identification of the oxidation product of thiosulfate in the pCL1 transformants of *Chl. limicola* DSMZ 245 could help clarify the biochemical mechanism of its thiosulfate utilization.

### H. A Potential Novel Complex: SoyYZ

The heterodimeric SoxYZ complex carries sulfur substrates on a conserved cysteine residue in the SoxY subunit (section III.G; Quentmeier and Friedrich, 2001). The *soxYZ* gene cluster has been duplicated in four GSB and is here denoted *soyYZ* (Table 2). (In *Chl. limicola* DSMZ 245, SoyY and SoyZ have the accession numbers EAM43192 and EAM43152, respectively.) A signal sequence at the amino termini of the SoyY sequences suggests that, like SoxYZ, SoyYZ is a periplasmic complex. Neither SoxZ nor SoyZ has a signal sequence, and both are probably transferred across the cytoplasmic membrane as part of complexes with SoxY or SoyY, respectively. The presence of *soyYZ* does not correlate with thiosulfate utilization (Table 2). In all GSB

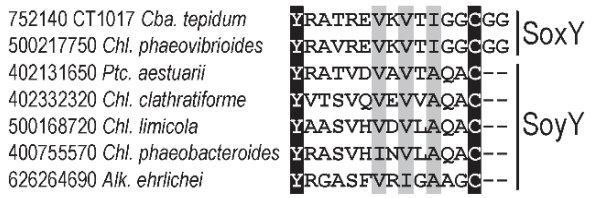


*Fig. 6*. Alignment of the C-terminal region of two SoxY proteins and all currently known SoyY proteins.

that have *soyYZ*, these genes are located immediately upstream of the *fccAB* genes in an apparent operon. Therefore, it is attractive to propose that SoyY and SoyZ form a complex in the periplasm that carries a sulfur substrate and that this complex reacts with the periplasmic FccAB flavocytochrome *c*. However, not all GSB that encode *fccAB* also encode *soyYZ*.

In most organisms of all taxonomic affiliations that have SoxY, the sulfur-substrate-carrying cysteine residue of SoxY is located at the C-terminus within the motif GGC($G_{1-2}$)–COOH. SoyY differs from all known examples of SoxY by having a C-terminus in which the putative sulfur-substrate-binding cysteine residue is the terminal residue (Fig. 6). The proximity of the C-terminal carboxyl group and the thiol group of the substrate-carrying cysteine residue in SoyY is likely to affect the chemistry at this site in a manner that does not occur in SoxY. If this is the case, this might explain the evolution of this particular motif in SoyY. In GSB the conserved motif in SoyY is VXAQAC-COOH. The *soyY* gene has only been found in one other organism other than GSB: the anaerobic, sulfide-oxidizing, chemoautotrophic *Alkalilimnicola ehrlichei* MLHE-1, which based on ribosomal RNA phylogeny is closely related to PSB of the *Ectothiorhodospiraceae* family. In *A. ehrlichei*, *soyY* (EAP35245) is located upstream of a *soxZ* homolog in a cluster of genes related to dimethyl sulfoxide utilization and cytochrome *c* biogenesis.

## IV. Other Enzymes Related to Sulfur Compound Oxidation

### A. RuBisCO-Like Protein

The enzyme RuBisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) catalyzes the key step in the Calvin–Benson–Bassham $CO_2$ fixation pathway in many phototrophic and chemotrophic organisms (Tabita, 1999). However, genome sequencing has revealed that some bacteria and archaea contain homologs of RuBisCO, called RuBisCO-like proteins (RLPs), which do not have the same enzymatic activity as *bona fide* RuBisCO. For example, in *Bacillus subtilis* RLP functions as a 2, 3-diketomethythiopentyl-1-phosphate enolase in the methionine salvage pathway of this organism (Ashida et al., 2003). All GSB genomes sequenced to date contain an RLP, but they do not have other recognizable genes for a methionine salvage pathway. A mutant of *Cba. tepidum* TLS lacking RLP (CT1772) has a pleiotropic phenotype with increased levels of oxidative stress proteins and defects in photopigment content, photoautotrophic growth rate, carbon fixation rates, and the ability to oxidize thiosulfate and elemental sulfur (Hanson and Tabita, 2001, 2003). Notably, sulfide oxidation is not affected in the *rlp* mutant of *Cba. tepidum* TLS. Hanson and Tabita subsequently suggested that RLP is involved in the biosynthesis of a low-molecular-weight thiol, which is essential for oxidation of thiosulfate and elemental sulfur. The possible role of such a hypothetical thiol as a carrier of sulfane sulfur is illustrated in Fig. 2. However, the function of GSB RLP is probably not limited to oxidation of inorganic sulfur compounds because *Chl. ferrooxidans* contains an RLP very similar to the RLP in other GSB, even though this organism cannot grow on inorganic sulfur compounds and does not contain genes thought to be involved in oxidation of thiosulfate (*sox* genes) and elemental sulfur (*dsr* genes) (Table 2).

### B. Polysulfide-Reductase-Like Complexes

Three types of complexes, here denoted polysulfide-reductase-like complex 1, 2, and 3 (PSRLC1, PSRLC2, and PSRLC3), with sequence similarity to the characterized polysulfide reductase (PSR) in *Wolinella succinogenes* (Krafft et al., 1992) are found in the genome sequences of GSB (Table 2). The *W. succinogenes* PSR is encoded by the *psrABC* genes and consists of two periplasmic subunits, a molybdopterin-containing PsrA subunit and a [4Fe–4S]-cluster-binding PsrB subunit, and a membrane-anchoring PsrC

subunit that binds an isoprenoid quinone and exchanges electrons with PsrB. In *W. succinogenes*, PSR and a hydrogenase allow respiration on polysulfide using $H_2$ as electron donor. However, homologs of PSR are also involved in metabolizing thiosulfate, tetrathionate, and other inorganic and organic compounds. Thus, the function of the PSR-like complexes in GSB cannot easily be established from sequence analysis alone.

Similar to the case of *W. succinogenes* PSR, polysulfide reductase-like complex 1 (PSRLC1, comprising CT0494, CT0495, and CT0496 in *Cba. tepidum* TLS) and PSRLC2 (comprising 400748340, 400748350, and 400748360 in *Chl. phaeobacteroides* DSM 266) are encoded by three genes. For both PSRLC1 and PSRLC2, the PsrA-like subunits with the catalytic site, have a Tat signal sequence and thus should be translocated into the periplasm. Homologs of PSRLC1 and PSRLC2 are found in many other organisms; for example, *Carboxydothermus hydrogenoformans* has a PSRLC1 homolog that has an overall amino acid sequence identity of approximate 50% with the PSRLC1 of *Cba. tepidum* TLS.

Two genes encode PSRLC3 in GSB. One is homologous with *psrA* (400751650 in *Chl. phaeobacteroides* DSMZ 266), and the other is homologous to a fusion of *psrB* and *psrC* (400751660 in *Chl. phaeobacteroides* DSMZ 266). Sequence analysis of the PsrBC-like subunit of PSRLC3 suggests that the PsrC-like domain has an orientation in the cytoplasmic membrane that is opposite that of the PsrC subunit of *W. succinogenes* PSR such that the PsrB-like domain of PSRLC3 is in the cytoplasm. In addition, the PsrA-like subunit of PSRLC3 does not have any obvious signal sequence. Thus, the catalytic PsrA-like catalytic subunit and the PsrB-like domain of PSRLC3 are probably located in the cytoplasm. Interestingly, the genes in GSB encoding PSRLC3 are immediately upstream of the *dsr* gene cluster. It is therefore an attractive possibility that PSRLC3 is involved in cytoplasmic oxidation of the sulfite produced by the Dsr system (section III.C). If so, PSRLC3 could provide all of the Dsr-containing GSB strains that lack the putative Sat-Apr-Qmo sulfite oxidation system (sections III.D–F, Fig. 2, Table 2) with a means to oxidize sulfite. Many known and putative prokaryotic sulfite oxidases are thought to be distantly related molybdopterin-containing enzymes that oxidize sulfite directly to sulfate (Kappler and Dahl, 2001). However, PSRLC3 is not widespread among other organisms, but a homologous complex with an overall amino acid sequence identity of approximate 50% is found in *Chloroflexus aurantiacus*, *Roseiflexus* sp. RS-1, and a few members of the high-GC *Firmicutes*.

### C. Sulfhydrogenase-Like and Heterodisulfide-Reductase-Like Complexes

A putative cytoplasmic αβγδ-heterotetrameric, bi-directional hydrogenase, which resembles *Pyrococcus furiosus* hydrogenase II that catalyzes $H_2$ production, $H_2$ oxidation, as well as the reduction of elemental sulfur and polysulfide to sulfide (Ma et al., 2000), is present in all sequenced GSB genomes, except those of *Chl. phaeovibrioides* DSM 265 and *Chl. luteolum* DSM 273. The genes encoding this putative sulfhydrogenase form a conserved *hyd1* cluster, *hydB1G1DA* (*CT1891–CT1894* in *Cba. tepidum* TLS), except in *Chl. chlorochromatii* CaD3 in which the genes are split into two clusters, *hydB1G1* and *hydDA*. Since *Cba. tepidum* TLS is unable to grow on $H_2$, these genes apparently do not confer the ability to oxidize large amounts of $H_2$. Likewise, the presence of this enzyme in *Chl. ferrooxidans* DSMZ 13031 and its absence from *Chl. phaeovibrioides* DSM 265 and *Chl. luteolum* DSM 273 suggests that its primary role is not related to elemental sulfur or polysulfide metabolism.

There are two types of complexes with sequence homology to heterodisulfide reductases encoded in the genomes of the sequenced GSB. One is the Qmo complex, which is probably involved in intracellular sulfite oxidation as discussed above (section III.F). The other is encoded by genes that form a conserved cluster with genes encoding a putative hydrogenase. This *hdr-hyd2* gene cluster, *hdrD-hdrA-orf1247-orf1248-hydB2-hydG2*, is conserved in seven of the sequenced strains (Fig. 4C). The *hdrD* gene in GSB is a fusion of the *hdrC* and *hdrB* genes found in other organisms. As with the Hyd1 complex mentioned above, the presence of Hdr-Hyd2 in *Chl. ferrooxidans* DSMZ 13031 and its absence from two other GSB strains,

suggests that its presence is not essential in elemental sulfur or polysulfide metabolism.

## V. Non-Sulfurous Compounds Oxidized for Growth

### A. Hydrogen

Many strains of GSB can grow on $H_2$ as electron donor (Overmann, 2000; Garrity and Holt, 2001). In general, cultures growing on $H_2$ need to be supplemented with a small amount of a reduced sulfur compound (such as sulfide or thiosulfate), probably to fulfill biosynthetic needs. This is not the case for strains capable of assimilatory sulfate reduction (section VI). The genomes of seven sequenced GSB strains contain a *hupSLCD* gene cluster that encodes a HupSL-type Ni–Fe uptake hydrogenase, a membrane-bound HupC cytochrome *b* subunit and the HupD maturation protein (Table 2). *Cba. tepidum* TLS contains a similar gene cluster, but in this organism *hupS* and a part of *hupL* have been deleted (Frigaard and Bryant, 2003). This deletion probably explains why *Cba. tepidum* TLS cannot grow on $H_2$ (T.E. Hanson and F.R. Tabita, personal communication).

Warthmann et al. (1992) found that *Chl. phaeovibrioides* DSMZ 265 produces $H_2$ and elemental sulfur from sulfide or thiosulfate under diazotrophic conditions in the light. When this strain was grown syntrophically with the sulfur-reducing bacterium *Desulfuromonas acteoxidans*, 3.1 mol (78% of the theoretical maximum) of $H_2$ were produced in a nitrogenase-dependent manner per mole of acetate consumed. This high efficiency in comparison to other GSB strains is possibly due to the absence of the *hupSLCD* genes from the genome of the DSMZ 265 strain (Table 2).

No GSB genome encodes genes homologous to *hoxEFUYH*, which together encode the subunits of a putative bidirectional NAD-reducing hydrogenase found in some cyanobacteria and the purple sulfur bacterium *Thiocapsa roseopersicina* (Tamagnini et al., 2002; Rakhely et al., 2004).

### B. Ferrous Iron

*Chlorobium ferrooxidans* DSMZ 13031 uses $Fe^{2+}$ as the sole electron donor for growth (Heising et al., 1999). This strain appears to have lost the ability to oxidize sulfur compounds because it does not grow on sulfide, elemental sulfur, or thiosulfate. This phenotype is largely confirmed by the absence of many genes related to oxidation of sulfur compounds in its genome (Table 2). Interestingly, *Chl. ferrooxidans* and *Chl. luteolum* DSMZ 273 are the only sequenced GSB whose genomes encode a bacterioferritin homolog (EAT59385 in *Chl. ferrooxidans*). Bacterioferritin binds heme *b* and non-heme iron and may be involved in the intracellular redox chemistry and storing of iron (Carrondo, 2003). *Chl. ferrooxidans* and *Chl. luteolum* DSMZ 273 also have a dicistronic operon encoding two *c*-type cytochromes that are not found in other GSB genomes. Although paralogs of the smaller cytochrome, a putative membrane-bound cytochrome of the $c_5/c_{555}$ family, are observed in other GSB genomes, the larger, cytochrome is uniquely found in these two strains (EAT58010 in *Chl. ferrooxidans*). The N-terminal region of this protein bears a single *c*-type, heme-binding sequence (CAACH), and this domain has weak sequence similarity to several other *c*-type cytochromes. Since *Chl. ferrooxidans* and *Chl. luteolum* DSMZ 273 differ from other sequenced GSB by having bacterioferritin and an identical cluster of assimilatory sulfate reduction and sulfate permease genes, it is possible that both strains can grow with $Fe^{2+}$ as the sole electron donor and by assimilatory sulfate reduction. Because the cytochromes mentioned above are also present only in *Chl. ferrooxidans* and *Chl. luteolum* DSMZ 273, they may be part of the $Fe^{2+}$ -oxidizing enzyme system.

### C. Arsenite

To our best knowledge, no GSB has been demonstrated to grow on arsenite. However, two GSB strains (*Chl. limicola* DSMZ 245 and *Prosthecochloris* sp. BS1) contain an enzyme consisting of a large molybdopterin-binding subunit and a small Rieske-type [2Fe–2S]-cluster-binding subunit (EAM42933 and EAM42934 in strain DSMZ 245, respectively) not found in any other genome-sequenced GSB strain. The small subunit contains a Tat signal peptide that presumably translocates the enzyme to the periplasm. The enzyme has high sequence similarity (an overall amino acid sequence identity of approximately 40%) with the

well-characterized arsenite oxidase from *Alcaligenes faecalis* (Anderson et al., 1992). This enzyme oxidizes arsenite ($AsO_3^{3-}$) to arsenate ($AsO_4^{3-}$) and donates the electrons to soluble, periplasmic cytochrome *c*. The presumed primary function of the enzyme in many organisms is to detoxify arsenite. Although the concentration of arsenite in most natural environments is low, the reduction of periplasmic cytochrome *c* in GSB would contribute to the photosynthetic electron transport and thus the growth of the organism. It is also possible that the enzyme in GSB oxidizes a different substrate (e.g. nitrite to nitrate).

## VI. Assimilatory Sulfur Metabolism

It is generally thought that GSB can not perform assimilatory sulfate reduction (Lippert and Pfennig, 1969). Nevertheless, *Chl. ferrooxidans* DSMZ 13031 grows with sulfate as the sole sulfur source and cannot utilize sulfide, thiosulfate, or elemental sulfur (Heising et al., 1999). Thus, *Chl. ferrooxidans* must be capable of assimilatory sulfate reduction. In agreement with this observation, the *Chl. ferrooxidans* genome encodes a single gene cluster that includes the assimilatory sulfate reduction genes *cysIHDNCG* and the sulfate permease genes *cysPTWA*, which are transcribed in opposite directions. These assimilatory sulfate reduction genes share a high degree of sequence similarity with those of the clostridia *Clostridium thermocellum* and *Desulfitobacterium hafniense*. However, sequence analyses show that the APS reductase encoded by *cysH* in *Chl. ferrooxidans* is the plant-type enzyme that uses APS and not PAPS as substrate. An identical *cys* gene cluster is observed in *Chl. luteolum* DSMZ 273, but these genes are not found in any other GSB genome. This raises the possibility that *Chl. luteolum* DSMZ 273 also is capable of assimilatory sulfate reduction and growth in the absence of reduced sulfur compounds using electron donors such as $H_2$ and $Fe^{2+}$.

## VII. Evolution of Sulfur Metabolism

Thiosulfate utilization by the Sox system in GSB is an interesting case study of lateral transfer of an ability that presumably confers a strong competitive advantage in certain natural environments. The Sox proteins are only present in some GSB strains and have phylogenies that are incongruent with that for ribosomal RNAs (Figs. 1 and 5). This indicates that the *sox* genes in GSB were not inherited vertically from a common ancestor. However, the Sox proteins from GSB form a monophyletic cluster in phylogenetic analyses (Fig. 5), which strongly implies that the currently known *sox* genes in GSB have only been laterally exchanged within the GSB. In addition, all eight genes in the *sox* gene cluster of GSB (Fig. 4A) have congruent phylogenies (data not shown). This suggests that all eight *sox* genes were transferred simultaneously as one conserved cluster to each recipient strain. How might such a transfer have occurred? Two GSB strains, strains DSMZ 273 and DSMZ 265, which are very closely related in terms of ribosomal RNA phylogeny and genome organization, differ in one important respect: the latter strain contains the *sox* gene cluster whereas the former strain does not (Table 2). Analysis of the genome sequences reveals that the *sox* cluster in strain DSMZ 265 resides on an island that contains four additional genes (Fig. 7). This island appears to have been inserted into a region of the genome in a recent ancestor that was not involved in sulfur metabolism. This ancestor was likely similar to strain DSMZ 273 and unable to use thiosulfate. The genes on the island include a transposase (EAO15044), an integrase (EAO15046), and an RNA-directed DNA polymerase (EAO15045),

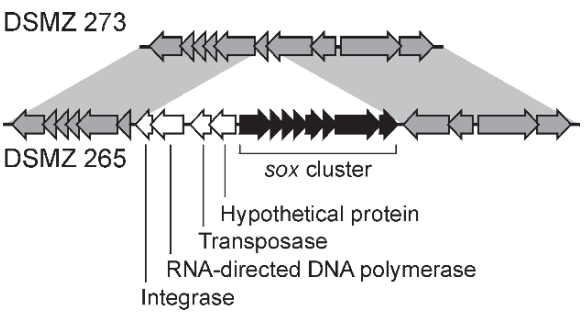


*Fig. 7*. Alignment of genomic regions from *Chl. luteolum* DSMZ 273, which cannot utilize thiosulfate, and *Chl. phaeovibrioides* DSMZ 265, which can utilize thiosulfate. Syntenic regions (i.e., regions having identical gene arrangements) are connected with gray trapezoids. Genes found in both organisms are shown in gray, *sox* genes are shown in black, and other genes are shown in white.

all of which are indicative of a mobile element. The RNA-directed DNA polymerase and the integrase are indicative of an RNA virus, and thus one possible scenario is that the island is a remnant structure derived from an RNA viral genome. The *sox* cluster could have been transferred into the viral genome by the transpose in a previous host and then integrated laterally into the genome of strain DSMZ 265. Highly similar RNA-directed DNA polymerases and integrases are found in several other GSB genomes in different genetic clusters, which suggests the existence of a GSB-specific RNA virus. To our knowledge, no virus of any kind that infects GSB has yet been isolated but there is no reason to believe such viruses do not exist.

Even though the GSB are closely related and exhibit limited apparent variation in physiology, it is obvious from genome sequence analyses that their gene contents are highly dynamic. For example, *Chl. clathratiforme* DSMZ 5477 and *Chl. ferrooxidans* DSMZ 13031 are closely related based on ribosomal RNA phylogeny (Fig. 1). However, whereas the former has the highest number of known and putative genes involved in oxidation of sulfur compounds among the GSB strains investigated, the latter has lost nearly all of these genes and in their place acquired the ability to reduce sulfate for assimilatory purposes (Table 2). On a similar note, *Chl. phaeobacteroides* DSMZ 266 has a single 3.1-Mbp chromosome and encodes roughly 1000 genes more than *Chl. phaeovibrioides* DSMZ 265 that only has a 2.0-Mbp chromosome (Table 1). These observations illustrate the dynamic structures of prokaryotic genomes and in addition demonstrate that organisms that are very closely related on the basis of their cellular core machinery nevertheless can have unexpected differences in physiology and life style.

## Acknowledgements

N.-U.F. gratefully acknowledges support from the Danish Natural Science Research Council (grant 21–04–0463). D.A.B. gratefully acknowledges support for genomics studies from the United States Department of Energy (grant DE-FG02–94ER20137) and the National Science Foundation (grant MCB-0523100).

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