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Molecular analysis of the distribution and phylogeny of the *soxB* gene among sulfur-oxidizing bacteria – evolution of the Sox sulfur oxidation enzyme system

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Summary

The *soxB* gene encodes the SoxB component of the periplasmic thiosulfate-oxidizing Sox enzyme complex, which has been proposed to be widespread among the various phylogenetic groups of sulfur-oxidizing bacteria (SOB) that convert thiosulfate to sulfate with and without the formation of sulfur globules as intermediate. Indeed, the comprehensive genetic and genomic analyses presented in the present study identified the *soxB* gene in 121 phylogenetically and physiologically divergent SOB, including several species for which thiosulfate utilization has not been reported yet. In first support of the previously postulated general involvement of components of the Sox enzyme complex in the thiosulfate oxidation process of sulfur-storing SOB, the *soxB* gene was detected in all investigated photo- and chemotrophic species that form sulfur globules during thiosulfate oxidation (*Chromatiaceae*, *Chlorobiaceae*, *Ectothiorhodospiraceae*, *Thiothrix*, *Beggiatoa*, *Thiobacillus*, invertebrate symbionts and free-living relatives). The SoxB phylogeny reflected the major 16S rRNA gene-based phylogenetic lineages of the investigated SOB, although topological discrepancies indicated several events of lateral *soxB* gene transfer among the SOB, e.g. its independent acquisition by the anaerobic anoxygenic phototrophic lineages from different chemotrophic donor lineages. A putative scenario for the proteobacterial origin and evolution of the Sox enzyme system in SOB is presented considering the phylogenetic, genomic (*sox* gene cluster composition) and geochemical data.

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Introduction

The sulfur compound thiosulfate has been suggested to fulfil a key role in the biological sulfur cycle in nature (Joergensen and Nelson, 2004; Zopfi *et al.*, 2004). A variety of photo- and chemotrophic sulfur-oxidizing prokaryotes (SOP) are able to use thiosulfate besides sulfide and sulfur as electron donor for their photosynthetic and respiratory energy-generating systems (Brune, 1995; Nelson and Fisher, 1995; Kelly *et al.*, 1997; Imhoff, 1999; 2001a,b; 2003; Brüser *et al.*, 2000; Robertson and Kuenen, 2002; Kletzin *et al.*, 2004; Takai *et al.*, 2005). In consequence of the phylogenetic and physiological diversity of SOP, several different enzymatic systems and pathways appear to be involved in the dissimilatory oxidation of thiosulfate. While the thiosulfate-converting enzymes of the archaeal sulfur oxidizers, e.g. *Acidianus ambivalens* (Kletzin *et al.*, 2004), represent a convergently evolved system, at least three thiosulfate oxidation pathways are postulated to exist in the sulfur-oxidizing bacteria (SOB) (Kelly *et al.*, 1997; Brüser *et al.*, 2000; Friedrich *et al.*, 2001; 2005). (i) The thiosulfate degradation process via polythionate intermediates involves the enzymes thiosulfate dehydrogenase and tetrathionate hydrolase and appears to be common in chemotrophic SOB living in extreme habitats, such as *Acidithiobacillus*, *Thermothiobacillus* and *Halothiobacillus* (Pronk *et al.*, 1990; Meulenberg *et al.*, 1993; Kelly *et al.*, 1997); in addition, some *Pseudomonas* and *Halomonas* species use the formation of tetrathionate from thiosulfate as supplemental energy source (Sorokin, 2003). However, no conclusive model for the formerly termed ‘tetrathionate pathway’ exists and the central role of tetrathionate has recently been disputed (Brüser *et al.*, 2000; and references therein). In addition, a different model not involving tetrathionate has been developed for the oxidation of elemental sulfur in acidophilic SOB (Rohwerder and Sand, 2003). (ii) The multienzyme complex system (Sox)-mediated pathway has been demonstrated to operate in photo- and chemotrophic *Alphaproteobacteria* that convert thiosulfate to sulfate without sulfur globule formation as free intermediate

(Mukhopadhyaya *et al*., 2000; Appia-Ayme *et al*., 2001; Friedrich *et al*., 2001; Kappler *et al*., 2001). The current model of the Sox enzyme system comprises the four periplasmic complexes SoxXA, SoxYZ, SoxB and $Sox(CD)_2$ that catalyse the thiosulfate oxidation according to the following mechanism. First, the SoxXA complex oxidatively couples the sulfane sulfur of thiosulfate to a SoxY-cysteine-sulfhydryl group of the SoxYZ complex from which the terminal sulfone group is subsequently released by the activity of the SoxB component. Subsequently, the sulfane sulfur of the residual SoxY-cysteine persulfide is further oxidized to cysteine-S-sulfate by the $Sox(CD)_2$ sulfur dehydrogenase complex from which the sulfonate moiety is again hydrolysed off by SoxB, thereby restoring SoxYZ; each of the previous proteins alone is catalytically inactive (Friedrich *et al*., 2001; 2005). The primary structure of the SoxB is about 30% identical to zinc-containing 5′-nucleotidases; however, besides its essential enzymatic activity as sulfate thioesterase component in the Sox enzyme system, no other *in vivo* function has been reported for this monomeric, dimanganese-containing protein (Epel *et al*., 2005). (iii) The branched thiosulfate oxidation pathway was postulated to operate in those bacteria that form sulfur globules during thiosulfate oxidation. This pathway proceeds via the interaction of two spatially separated enzyme systems; the sulfone sulfur is rapidly converted to sulfate in the periplasm, whereas the sulfane sulfur accumulates as intracellularly or periplasmically deposited sulfur [S^0] before further oxidation by cytoplasmic enzymes. Previously, the thiosulfate oxidation was suggested to be initiated by the activity of periplasmic thiosulfate reductases or rhodaneses via a reductive cleavage of the molecule (Brune, 1995; Brüser *et al*., 2000). Increasing experimental data indicate that components of the Sox enzyme system are instead involved in the initial step of the branched thiosulfate oxidation pathway of some sulfur-storing bacteria (Hanson and Tabita, 2003; Friedrich *et al*., 2005; Hensen *et al*., 2006). In consequence, the oxidation of reduced inorganic sulfur compounds via components of the Sox enzyme system was postulated to be a widespread mechanism among the SOB (Friedrich *et al*., 2001; 2005; Hensen *et al*., 2006). However, a comprehensive investigation of the phylogentically diverse SOB had not been performed to confirm this proposal. In first support, Petri and coworkers (2001) proved the presence of SoxB encoding genes in eight thiosulfate-utilizing reference strains from the *Alpha-, Beta-* and *Gammaproteobacteria* as well as *Chlorobia* lineage. Their presented SoxB phylogenetic tree was based on a limited dataset not including representatives of several major SOB lineages, e.g. *Chromatiaceae*, *Ectothiorhodospiraceae*, *Thiotrichaceae*, invertebrate symbionts and their free-living relatives, as well as *Sulfurimonas denitrificans* (Takai *et al*., 2006).

To evaluate the former postulation by Friedrich and coworkers, the previously published polymerase chain reaction (PCR) assays (Petri *et al*., 2001) were used to investigate the *soxB* distribution among 116 different photo- and chemotrophically SOB strains considering especially the thiosulfate-oxidizing, sulfur-storing species. The comparison of the SoxB- and 16S rRNA gene-based tree topologies indicated the occurrence of several putative lateral gene transfer (LGT) events of the *soxB* gene among the SOB. A potential scenario for the origin and evolution of the microbial thiosulfate oxidation processes is presented in context with the gene composition of the *sox* gene loci in SOB genomes and the geochemical data.

Results

Amplification of soxB *genes by PCR from SOB*

The PCR-based analysis confirmed the presence of the *soxB* gene for 50 different photo- and chemotrophic sulfur-oxidizing species from 116 investigated reference strains (see Table 1 for details of PCR results; potential contamination of the examined reference strains could be excluded by 16S rRNA gene-based analyses). In general, the amplification with soxB693F/soxB1446R and soxB693F/soxB1164B (Table 2) resulted in single, correct-sized PCR products (~750 bp and ~470 bp, respectively), whereas the primer pair soxB432F/soxB1446R (Table 2) frequently generated two amplicons of nearly identical length (~1000 bp) with the consequence of ambiguous direct sequencing results. Analysis of genome data revealed that the highly degenerated primers are complementary to the target sites of *Chlorobiaceae*, *Betaproteobacteria* and most *Gamma-* and *Alphaproteobacteria soxB* sequences. Therefore, the negative amplification results obtained from several proven SOB species of, e.g. *Chromatiaceae* and *Chlorobiaceae* with the three different primer sets were most probably not caused by inhibited primer annealing but are indicative for the absence of this gene in the respective strain (see Table 1). The results of the PCR-based analysis are supported by: (i) the Southern blot assays resulting in no hybridization signal for the examined *Chlorobiaceae* species of the subclusters 2a and 3b (except *Chlorobium limicola* DSM 1855) irrespective of *soxB* probes used (see Table 3; probe specificities and stringency of hybridization conditions verified by the negative hybridization results obtained with genomic DNA from non-thiosulfate-oxidizing *Desulfomicrobium baculatum*); and (ii) genome data (Table 4). In contrast, the target sites of *Hyphomicrobiaceae* and *Rhodopseudomonas* spp. (*Alphaproteobacteria*), *Thiomicrospira crunogena* and '*Candidatus* Ruthia magnifica' (*Gammaproteobacteria*), as well as *S. denitrificans* (*Epsilonproteobacteria*), harboured two or more mismatches at the 3′-end

Table 1. Polymerase chain reaction (PCR) amplification results of *soxB* gene fragments from genomic DNA of sulfur-oxidizing reference strains.

Species[a]	Strain[b]	PCR product obtained with primer set[c]			Length of obtained *soxB* sequence	GenBank accession no. *soxB*
		soxB432F soxB1446B	soxB693F soxB1446B	soxB693F soxB1164B		
Archaea						
Crenarchaeota phylum, *Thermoprotei*						
Sulfolobaceae						
Acidianus ambivalens	3772	–	–	n.d.	–	–
Metallosphaera sedulae[d]	5348^{T}	–	–	n.d.	–	–
Metallosphaera prunae[d]	10039	–	–	n.d.	–	–
Sulfolobus metallicus[d]	6482	–	–	n.d.	–	–
Bacteria						
Chloroflexi phylum, *Chloroflexi*						
Chloroflexaceae						
Chloroflexus aggregans[d]	9485	–	–	n.d.	–	–
Chlorobi phylum, *Chlorobia*						
Chlorobiaceae						
1 *Prosthecochloris aestuarii*[e,d]	271^{T}	–	–	n.d.	–	–
Prosthecochloris sp.[e,d]	*2K*	–	–	n.d.	–	–
Prosthecochloris vibrioforme[e,d]	260	–	–	n.d.	–	–
Prosthecochloris vibrioforme[e,d]	1678	–	–	n.d.	–	–
2a *Chlorobium luteolum*[e,d]	273^{T}	–	–	n.d.	–	–
Chlorobium luteolum[e,d]	262	–	–	n.d.	–	–
2b *Chlorobium phaeovibrioides*[e,d]	269^{T}	–	–	n.d.	–	–
Chlorobium phaeovibrioides[e,f]	265	+	+	n.d.	database	AJ294321
Chlorobium phaeovibrioides[e,d]	261	–	–	n.d.	–	–
Chlorobium phaeovibrioides[e,d]	270	–	–	n.d.	–	–
3a *Chlorobium phaeobacteroides*[e,d]	266^{T}	–	–	n.d.	–	–
Chlorobium clathratiforme[e]	5477^{T}	+	+	n.d.	database	AJ294323
'Chlorobium ferrooxidans'[d]	13031^{T}	–	–	n.d.	–	–
3b *Chlorobium limicola*[e,d]	245^{T}	–	–	n.d.	–	–
Chlorobium limicola[e]	246	–	–	n.d.	–	–
Chlorobium limicola[e]	*2323*	+	+	+	1002	EF618588
Chlorobium limicola[e,f]	1855	+	+	n.d.	1026	EF618591
Chlorobium limicola[e]	257	+	+	+	1026	EF618579
Chlorobium limicola[e,d]	247	–	–	n.d.	–	–
Chlorobium limicola[e,d]	248	–	–	n.d.	–	–
4a *Chlorobaculum parvum*[e]	263^{T}	+	+	n.d.	database	AJ294320
Chlorobaculum parvum[e]	*2352*	+	+	n.d.	1026	EF618589
4b *Chlorobaculum limnaeum*[e,f]	1677	+	+	n.d.	1026	EF618590
Chlorobaculum thiosulfatiphilum[e]	249^{T}	n.d.	n.d.	n.d.	database	AAL68888
Chlorobaculum thiosulfatiphilum[e]	*2322*	+	+	+	959	EF618587
Proteobacteria phylum, *Alphaproteobacteria*						
Rhodospirillaceae						
Rhodospirillum photometricum	122^{T}	+	–	n.d.	918	EF618569
Rhodobacteraceae						
Rhodothalassium salexigens	2132^{T}	±	±	n.d.	679	EF618585
Rhodovulum adriaticum	2781	±	+	n.d.	972	EF618592
Rhodovulum sulfidophilum	1374^{T}	+	+	n.d.	database	AAF99435
Bradyrhizobiaceae						
Rhodoblastus acidophilus	137^{T}	–	–	n.d.	–	–
Hyphomicrobiaceae						
Blastochloris viridis[d]	133^{T}	–	–	n.d.	–	–
Rhodobiaceae						
Rhodobium marinum[d]	2698^{T}	–	–	n.d.	–	–
Proteobacteria phylum, *Betaproteobacteria*						
Hydrogenophilaceae						
Thiobacillus aquaesulis	4255^{T}	+	+	n.d.	999	EF618597
Thiobacillus denitrificans	12475^{T}	+	+	n.d.	981	EF618607
Thiobacillus denitrificans	739	n.d.	n.d.	n.d.	–	–
Thiobacillus denitrificans	807	n.d.	n.d.	+	501	EF618581
Thiobacillus plumbophilus	6690^{T}	+	+	n.d.	765	EF618604
Thiobacillus thioparus	505^{T}	+	n.d.	n.d.	database	AJ294326
Neisseriaceae						
Aquaspirillum sp. strain D-412[d]	–	–	–	n.d.	–	–
Aquaspirillum sp. strain D-415[d]	–	–	–	n.d.	–	–

Table 1. *cont.*

Species[a]	Strain[b]	PCR product obtained with primer set[c]			Length of obtained *soxB* sequence	GenBank accession no. *soxB*
		soxB432F soxB1446B	soxB693F soxB1446B	soxB693F soxB1164B		
Proteobacteria phylum, *Gammaproteobacteria*						
Chromatiaceae						
Allochromatium minutissimum	1376^{T}	+	+	n.d.	1008	EF618582
Allochromatium vinosum	180^{T}	±	+	n.d.	1017	EF618570
Allochromatium warmingii[d]	173^{T}	–	–	n.d.	–	–
Chromatium okenii[e]	*6010*	±	+	n.d.	729	EF618602
Halochromatium glycolicum	11080^{T}	+	+	n.d.	966	EF618605
Halochromatium salexigens	4395^{T}	+	+	n.d.	1018	EF618598
Isochromatium buderi[d]	176^{T}	–	–	n.d.	–	–
Lamprocystis purpurea[e]	4197^{T}	+	±	n.d.	919	EF618595
Marichromatium gracile	203^{T}	±	+	n.d.	1017	EF618572
Marichromatium purpuratum	1591^{T}	+	+	n.d.	1017	EF618584
Rhabdochomatium marinum	5261^{T}	±	–	+	713	EF618601
Thermochromatium tepidum[d]	3771^{T}	–	–	–	–	–
Thiocapsa pendens	236^{T}	+	+	–	990	EF618577
Thiocapsa rosea[e]	235^{T}	±	n.d.	–	–	–
Thiocapsa roseopersicina	217^{T}	+	+	n.d.	1023	EF618576
Thiocapsa roseopersicina[e]	*4210*	+	+	n.d.	1023	EF618596
Thiococcus pfennigii[e,d]	226^{T}	–	–	n.d.	–	–
Thiococcus pfennigii[d]	227	–	–	–	–	–
Thiococcus pfennigii[d]	228	–	–	–	–	–
Thiocystis gelatinosa[f]	215^{T}	+	n.d.	–	950	EF618575
Thiocystis violacea	207^{T}	+	n.d.	+	984	EF618573
Thiocystis violacea	214	+	+	n.d.	1008	EF618574
Thiocystis violascens	198^{T}	+	+	+	987	EF618571
Thiodictyon bacillosum[e,d]	234^{T}	–	n.d.	n.d.	–	–
Thiodictyon sp. strain F4[d]	–	–	–	–	–	–
Thiohalocapsa halophila	6210^{T}	+	n.d.	+	981	EF618603
Thiolamprovum pedioforme	3802^{T}	+	n.d.	+	993	EF618593
Thiorhodococcus minor	11518^{T}	+	n.d.	+	1029	EF618606
Thiorhodovibrio winogradskyi[d]	6702^{T}	–	–	–	–	–
Ectothiorhodospiraceae						
Ectothiorhodospira mobilis[g]	4180	+	+	n.d.	1011	EF618594
Ectothiorhodospira shaposhnikovii[f]	243^{T}	+	+	n.d.	1011	EF618578
Halothiobacillaceae						
Halothiobacillus hydrothermalis	7121^{T}	–	+	n.d.	database	AJ294325
Halothiobacillus kellyi	13162^{T}	+	+	n.d.	954	EF618609
Halothiobacillus neapolitanus	581^{T}	+	+	n.d.	database	AJ294332
Thiovirga sulfuroxydans sp. strain A7	–	+	+	n.d.	735	EF618610
Thiotrichaceae						
Beggiatoa alba	1416^{T}	+	+	n.d.	858	EF618583
Beggiatoa leptomitiformis strain D-401[d]	–	n.d.	n.d.	n.d.	–	–
Beggiatoa leptomitiformis strain D-402	–	n.d.	n.d.	n.d.	–	–
Leucothrix mucor	2157^{T}	–	+	+	465	EF618586
Leucothrix mucor[f]	621	–	+	+	669	EF618580
Macromonas bipunctata strain D-408[d]	–	±	–	n.d.	–	–
Thiothrix nivea	5205^{T}	n.d.	+	n.d.	738	EF618600
Thiothrix sp.	12730	n.d.	+	n.d.	765	EF618608
Piscirickettsiaceae						
Thiomicrospira frisia	12351^{T}	–	–	–	–	–
Thiomicrospira kuenenii	12350^{T}	–	–	–	–	–
Thiomicrospira sp.	13163	–	n.d.	–	–	–
Thiomicrospira sp.	13164	–	n.d.	–	–	–
Thiomicrospira sp.	13189	–	n.d.	–	–	–
Thiomicrospira sp.	13190	–	n.d.	–	–	–
Uncertain affiliation						
'Thiobacillus prosperus'	5130^{T}	+	n.d.	–	447	EF618599
Invertebrate symbionts and free-living relatives						
Bathymodiolus azoricus symbiont	–	–	–	–	–	–
Bathymodiolus brevior symbiont	–	–	–	–	–	–
Bathymodiolus thermophilus symbiont	–	–	–	n.d.	–	–
Calyptogena magnifica symbiont	–	–	–	n.d.	–	–
Ifremeria nautilei symbiont[f]	–	+	+	n.d.	766	EF618614

Table 1. *cont.*

Species[a]	Strain[b]	PCR product obtained with primer set[c]			Length of obtained *soxB* sequence	GenBank accession no. *soxB*
		soxB432F soxB1446B	soxB693F soxB1446B	soxB693F soxB1164B		
Inanidrilus exumae symbiont[d]	–	–	–	n.d.	–	–
Inanidrilus leukodermatus symbiont[d]	–	–	–	n.d.	–	–
Inanidrilus makropetalos symbiont[d]	–	–	–	n.d.	–	–
Oasisia sp. symbiont[d]	–	–	–	n.d.	–	–
Riftia pachyptila symbiont	–	+	+	n.d.	756	EF618617
sulfur-oxidizing bacterium OAII2	–	+	n.d.	n.d.	993	EF618611
sulfur-oxidizing bacterium OBII5	–	+	+	n.d.	975	EF618612
sulfur-oxidizing bacterium ODIII5	–	–	–	n.d.	–	–
sulfur-oxidizing bacterium ODI4	–	+	n.d.	+	936	EF618613
sulfur-oxidizing bacterium NDII1.2	–	–	n.d.	+	501	EF618616
sulfur-oxidizing bacterium ‘manganese crust’	–	+	n.d.	n.d.	972	EF618615
Proteobacteria phylum*, Epsilonproteobacteria*						
Helicobacteraceae						
Sulfurimonas denitrificans	1251[T]	–	–	–	database	YP_392780
Spirochaeta phylum*, Spirochaetes*						
Spirochaetaceae						
Spirochaeta sp. strain P[d]	–	±	±	n.d.	–	–
Spirochaeta sp. strain BM[d]	–	±	±	n.d.	–	–
Spirochaeta sp. strain M-6[f]	–	±	±	n.d.	927	EF618568

a. Taxonomic classification of investigated SRP species according to the taxonomic outline of the prokaryotes, Bergey’s Manual of Systematic Bacteriology, 2nd edition, release 5.0 May 2004 (http://dx.doi.org/10.1007/bergeysoutline); genomic DNA of sulfur-oxidizing reference strains signed with **e** were received from the culture collection of J. Imhoff, University of Kiel.

b. DSM identification numbers of investigated species (laboratory-internal numbers of culture collection from J. Imhoff in italic type); (–) not deposited in a culture collection; T, type strain.

c. *soxB* gene PCR results obtained from genomic DNA of sulfur-oxidizing reference strains are summarized with the following abbreviations: (–) no amplicon; (+) correct-sized amplicon; (±) correct-sized amplicon with byproducts; (n.d.) PCR amplification not determined.

d. Thiosulfate-oxidizing ability not experimentally proven for respective species (Brune, 1995; Nelson and Fisher, 1995; Brinkhoff *et al.*, 1999; Howarth *et al.*, 1999; Imhoff, 1999; 2001a,b,c; 2003; Kelly and Wood, 2000; Kuever *et al.*, 2002; Cavanaugh *et al.*, 2004; Dubinina *et al.*, 2004; Kletzin *et al.*, 2004; Teske and Nelson, 2004; Takai *et al.*, 2006).

f. Thiosulfate-oxidizing ability of *soxB* gene-harbouring SOB species not experimentally proven (Nelson and Fisher, 1995; Imhoff, 1999, 2001a; 2003; Kuever *et al.*, 2002; Cavanaugh *et al.*, 2004; Dubinina *et al.*, 2004; Teske and Nelson, 2004).

g. Uncertain taxonomic classification (synonym *Ectothiorhodospira marismortui*).

sequence position of one or both primers of the applied primer sets. While internally or at the 5′-end located, single mismatches have only a limited effect on the primer annealing efficiency (Kwok *et al.*, 1990; Simsek and Adnan, 2000), their position at the 3′-end of the primer sequence severely affects the PCR efficiency. In consequence, the *soxB* PCR primer combinations used will have failed to amplify gene fragments from certain examined genera, e.g. *Thiomicrospira* spp. and related symbionts of the Vesicomyid mussels and Mytilid clam, *S. denitrificans* and putatively *Rhodoblastus acidophilus*.

Phylogeny of sulfate thioesterase (SoxB) of SOB

The SoxB consensus tree presented in this work (Fig. 1) is based on 124 sequences obtained from genetic and genomic analyses (Tables 1 and 4). The integration of 50 novel SoxB partial sequences from sulfur-storing photo- and chemotrophic bacteria, e.g. *Chromatiaceae*, *Ectothiorhodospiraceae*, *Thiotrichaceae*, thiotrophic symbiont of invertebrates and their free-living relatives (Table 1) which were previously not considered (Petri *et al.*, 2001), allowed new insights into the evolutionary

Table 2. Polymerase chain reaction (PCR) primers used for amplification of *soxB* gene fragments.

Primer[a]	Sequence (in 5′→3′ direction)[b]							Primer binding site[c]
soxB432F	GA**Y**	GG**N**	GG**N**	GA**Y**	AC**N**	TGG		432–450
soxB693F	ATC	GG**N**	CA**R**	GC**N**	TT**Y**	CC**N**	TA	693–713
soxB1164B	AA**R**	TT**N**	CC**N**	CG**N**	CG**R**	TA		1181–1166
soxB1446B	CAT	GTC	**N**CC	**N**CC	**R**TG	**Y**TG		1446–1428

a. Source: Petri *et al.* (2001).

b. Degenerate positions are in boldface.

c. *soxB* primer binding sites are enumerated according to the nucleotide sequence of *Paracoccus denitrificans* str. GB 17 (GenBank accession no. CAA55824).

Table 3. Results of Southern blot assays with radioactively labelled *soxB*-specific probes and genomic DNA of sulfur-oxidizing and sulfate-reducing bacteria.

Genomic DNA of SOB and SRB species (*Eco*RI/*Hind*III digestion)	Strain[a]	Southern blot hybridization results with *soxB*-specific probe[b]			
		Chlorobium limicola 1855	*Chlorobium limicola* 257	*Chlorobium clathrathiforme* 5477	*Thiocapsa roseopersicina 4210*
Gammaproteobacteria					
Thiocapsa roseopersicina	217	±	±	±	++
Thiocapsa roseopersicina	*4210*[c]	±	±	±	++
Chlorobia					
Chlorobium limicola	245[c]	–	–	–	–
Chlorobium limicola	248[c]	–	–	–	–
Chlorobium limicola	1855[c]	++	++	+	±
Chlorobium luteolum	262[c]	–	–	–	–
Chlorobium luteolum	273[c]	–	–	–	–
Deltaproteobacteria					
Desulfomicrobium baculatum	4028	–	–	–	–

a. DSM identification numbers of investigated species (J. Imhoff laboratory-internal numbers are in italic type); cultures received from the culture collection of J. Imhoff are marked with c.
b. Quality of hybridization results summarized with the following abbreviations: (–) no hybridization (+) hybridization signal (++) strong hybridization signal.

path of *soxB* genes among SOB. The overall tree topology was congruent with the previous one based on a limited dataset of 13 validated SOB species (Petri *et al*., 2001). However, with respect to the improved species coverage, the enlarged database refined the resolution of the inter- and intrafamily relationships in the major SoxB lineages. Comparative analysis of the SoxB- and the 16S rRNA-based phylogenetic tree (Fig. 2; see also references Imhoff, 1999; 2001a,b,c; 2003; Kelly and Wood, 2000; Kuever *et al*., 2002; Cavanaugh *et al*., 2004; Buchan *et al*., 2005; Takai *et al*., 2006) revealed several topological discrepancies indicative for incorrect taxonomical classifications and even lateral *soxB* gene transfers among SOB (marked by letters in the trees). According to the SoxB phylogeny, the alphaproteobacterial *Rhodobacteraceae* and *Bradyrhizobiaceae* (Imhoff, 2001b) are not monophyletic (see distinct branching position of *Rhodobacteraceae* representatives *Stappia aggregata* and *Rhodothalassium salexigens* and the cluster formation of *Bradyrhizobiaceae* members), and *Rhodospirillum photometricum* (*Rhodospirillaceae*) is affiliated with *Rhodopseudomonas* spp. (*Bradyrhizobiaceae*). Indeed, the current taxonomical classification of *R. salexigens* and *S. aggregata* is also not well supported by the 16S rRNA gene-based phylogeny. Potential LGT events involving *Alphaproteobacteria* are indicated by the 16S rRNA gene-incongruent close relationships of (i) *Spirochaeta* sp. strain M-6 (Dubinina *et al*., 2004) to *Sulfitobacter* spp. (LGT a), and (ii) *Acidiphilium cryptum*, *Nitrobacter hamburgensis* and *Bradyrhizobium* spp. (*Alphaproteobacteria* II) to the *Gammaproteobacteria* (LGTs b and c). Interestingly, the latter xenologous cluster comprises species which harbour a second, non-LGT-affected *soxB* gene in their genomes (*Bradyrhizobium* spp.). The 16S rRNA gene-discordant affiliation of *Anaeromyxobacter dehalogenans* (*Deltaproteobacteria*) and *Thiovirga sulfuroxydans* strain A7 (*Gammaproteobacteria*) with the *Betaproteobacteria* points to further lateral transfers of *soxB* genes with the previous species as recipients (LGTs d and e). According to the SoxB tree, the *Gammaproteobacteria* were not monophyletic but formed at least four distinct SOB groups consisting of the *Thiotrichaceae*, '*Thiobacillus prosperus*', *Halothiobacillaceae*, free-living relatives of invertebrate symbionts and *Ectothiorhodospira* spp. (cluster I), the *Piscirickettsiaceae*, *Oceanospirillum* sp., *Beggiatoa alba*, invertebrate symbionts and *Chromatiaceae* (cluster II), the newly described *Congregibacter litoralis* (cluster III), and *Halorhodospira halophila* (cluster IV). The SoxB-proposed separate branching positions of *Thiothrix*/*Leucothrix* and *Beggiatoa* members are supported by the 16S rRNA gene-based phylogeny (Fig. 2) and point to their incorrect classification at the family level (*Thiotrichaceae*). According to the SoxB phylogeny, the *Chromatiaceae* and affiliated invertebrate symbionts are closest related to members of the *Piscirickettsiaceae* and *Oceanospirillum* (cluster II). The affiliation of the *Ectothiorhodospira* spp. with the *Halothiobacillaceae* (cluster I) while *H. halophila* formed a distinct lineage (cluster IV) is discordant to their close relationship based on the 16S rRNA phylogeny (*Ectothiorhodospiraceae*) and indicates independent lateral transfers of *soxB* genes to the anaerobic anoxygenic phototrophic lineages (including the symbionts) (LGTs f to h). The 16S rRNA gene-incongruent affiliation of the *Chlorobiaceae* with the *Gammaproteobacteria* cluster II points also to a lateral *soxB* acquisition of the green sulphur bacteria (LGT i). The detailed comparison of the relative branching order within the *Chlorobiaceae*

Table 4. Presence of *sox*, *sor*, *apr* and *dsr* homologues coding for the Sox enzyme system (SoxXAYZBCD), sulfite dehydrogenase (SorAB, *Starkeya novella*), dissimilatory APS reductase (AprBA) and sulfite reductase (DsrAB) including its functionally associated transmembrane complex (DsrMKJOP) in genome sequences of *Bacteria* (the genomic arrangement is indicated by the GenBank accession numbers of the encoded proteins).

	Homologues present in genome sequences of *Bacteria*[b]							
	Sox				Sor	Apr	Dsr	
Species[a]	SoxXA	SoxYZ	SoxB	SoxCD	SorAB	AprBA	DsrAB	DsrMKJOP
Bacteria								
Aquificae phylum, *Aquificae*								
Aquificaceae								
Aquifex aeolicus str. VF5[c]	NP_214238; NP_214239	NP_214241; NP_214240	NP_214237	–	–	–	–	–
Deinococcus–Thermus phylum, *Deinococci*								
Thermaceae								
Thermus thermophilus str. HB8[c]	YP_144682/ YP_144684; YP_144681/ YP_144685	YP_144687; YP_144686	YP_144683	YP_144677; YP_144676	–	–	–	–
Thermus thermophilus str. HB27[c]	YP_005020/ YP_005022; YP_005019/ YP_005023	YP_005025; YP_005024	YP_005021	YP_005015; YP_005014	–	–	–	–
Chloroflexi phylum, *Chloroflexi*								
Chloroflexaceae								
Chloroflexus aggregans DSM 9485	–	–	–	–	–	–	–	–
Chloroflexus aurantiacus str. J-10-fl	–	–	–	–	–	–	–	–
Chlorobi phylum, *Chlorobia*								
Chlorobiaceae								
1a *Prosthecochloris aestuarii* str. DSM 271	–	–	–	–	–	–	+	+
2a *Chlorobium luteolum* str. DSM 273	–	–	–	–	–	–	+	+
2b Chlorobium phaeovibrioides str. DSM 265	ZP_00661606; ZP_00661604	ZP_00661605; ZP_00661603	ZP_00661601	–	–	–	+	+
3a *Chlorobium phaeobacteroides* str. DSM 266	–	–	–	–	–	–	+	+
Chlorobium phaeobacteroides str. BS1	–	–	–	–	–	+	+	+
Chlorobium clathratiforme str. DSM 5477	ZP_00588637; ZP_00588640	ZP_00588638; ZP_00588639	ZP_00588642	–	–	+	+	+
Chlorobium chlorochromatii str. CaD3	YP_380213; ZP_380216	YP_380214; ZP_380215	YP_380218	–	–	+	+	+
3b *Chlorobium limicola* strain DSM 245	–	–	–	–	–	–	+	+
4b *Chlorobaculum tepidum* str. ATCC 49652	NP_661908; NP_661911	NP_661909; NP_661910	NP_661913	–	–	+	+	+
Chlorobaculum thiosulfatophilum str. DSM 249	AAL68883; AAL68886[d]	AAL68884; AAL68885[d]	AAL68888[d]	–[d]	n.a.[d]	n.a.[d]	n.a.[d]	n.a.[d]
Proteobacteria phylum, *Alphaproteobacteria*								
SAR11-cluster								
Pelagibacter ubique str. HTCC1002	–	–	–	–	–	+	–	–
Pelagibacter ubique str. HTCC1062	–	–	–	–	–	+	–	–

Table 4. *cont.*

	Homologues present in genome sequences of *Bacteria*[b]							
	Sox				Sor	Apr	Dsr	
Species[a]	SoxXA	SoxYZ	SoxB	SoxCD	SorAB	AprBA	DsrAB	DsrMKJOP
SAR116-cluster								
Uncultured Alphaproteobacterium EBAC2C11	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.	n.a.
Rhodospirillaceae								
Magnetospirillum magneticum str. AMB-1	–	–	–	–	–	–	+	+
Magnetospirillum magnetotacticum str. MS-1[c]	–; ZP_00051298	ZP_00048026; ZP_00056090	ZP_00051299	ZP_00050505; –	ZP_00051098; ZP_00051120	–	+	+
Acetobacteraceae								
Acidiphilium cryptum str. JF-5[c]	ZP_01144910; ZP_01144907	ZP_01144909; ZP_01144908	ZP_01144905	ZP_01144912; ZP_01144911	ZP_01144296; ZP_01144295	–	–	–
Rhodobacteraceae						–	–	–
Dinoroseobacter shibae str. DFL 12[c]	ZP_01583271; ZP_01583268	ZP_01583270; ZP_01583269	ZP_01583267	ZP_01583266; ZP_01583265	–	–	–	–
Paracoccus denitrificans str. GB17	CAB94379; CAA55827[d]	CAB94380; CAB94381[d]	CAA55824[d]	CAA55829; CAA55825[d]	n.a.[d]	n.a.[d]	n.a.[d]	n.a.[d]
Paracoccus denitrificans str. PD1222	ZP_00628734; ZP_00628737	ZP_00628735; ZP_00628736	ZP_00628738	ZP_00628739; ZP_00628740	–	–	–	–
Rhodobacter sphaeroides str. 2.4.1	–	–	–	–	–	–	–	–
Rhodobacter sphaeroides str. ATCC 17025	ZP_00912867; ZP_00912864	ZP_00912866; ZP_00912865	ZP_00912863	ZP_00912862; ZP_00912861	–	–	–	–
Rhodobacter sphaeroides str. ATCC 17029	–	–	–	–	–	–	–	–
Roseobacter sp. str. MED193[c]	ZP_01055916; ZP_01055913	ZP_01055915; ZP_01055914	ZP_01055912	ZP_01055911; ZP_01055910	–	–	–	–
Roseobacter denitrificans str. Och 114[c]	YP_681830; YP_681833	YP_681831; YP_681832	YP_681834	YP_681835; YP_681836	–	–	–	–
Roseovarius nubinhibens str. ISM	ZP_00961298; ZP_00961295	ZP_00961297; ZP_00961296	ZP_00961294	ZP_00961293; ZP_00961292	–	–	–	–
Roseovarius sp. str. 217[c]	ZP_01037120; ZP_01037117	ZP_01037119; ZP_01037118	ZP_01037116	ZP_01037115; ZP_01037114	–	–	–	–
Rhodovulum sulfidophilum str. DSM 1374	AAF99431; AAF99434	AAF99432; AAF99433	AAF99435	AAF99436; AAF99437	–	–	–	–
Sagittula stellata str. E-37	ZP_01748363; ZP_01748360	ZP_01748362; ZP_01748361	ZP_01748359	ZP_01748358; ZP_01748357	–	–	–	–
Silicibacter pomeroyi str. DSS-3	YP_166245; YP_166248	YP_166246; YP_166247	YP_166249	YP_166250; YP_166251	–	–	–	–
Silicibacter sp. str. TM1040	–	–	–	–	–	–	–	–
Stappia aggregata str. IAM 12614[c]	ZP_01549051; ZP_01549054	ZP_01549052; ZP_01549053	ZP_01549055	ZP_01549056; ZP_01549057	–	–	–	–
Sulfitobacter sp. str. NAS-14.1	ZP_00963533; ZP_00963530/ ZP_00963374	ZP_00963532/ ZP_00963372; ZP_00963531/ ZP_00963373	ZP_00963529/ ZP_00963375	–/ ZP_00963377; ZP_00963526/ ZP_00963378	–	–	–	–
Sulfitobacter sp. str. EE-36	ZP_00956135; ZP_00956138	ZP_00956136; ZP_00956137	ZP_00956139	ZP_00956140; ZP_00956141	–	–	–	–

Uncertain phylogenetic affiliation								
Rhodobacterales bacterium str. HTCC 2654[c]	ZP_01014855; ZP_01014858	ZP_01014856; ZP_01014857	ZP_01014859	ZP_01014860; ZP_01014861	–	–	–	–
Rhodobacterales bacterium str. HTCC 2150[c]	ZP_01743249; ZP_01743246	ZP_01743248; ZP_01743247	ZP_01743245	ZP_01743243; ZP_01743242	–	–	–	–
Aurantimonadaceae								
Aurantimonas sp. str. SI85-9A1[c]	ZP_01225769; ZP_01225766	ZP_01225768; ZP_01225767	ZP_01225765	ZP_01225764; ZP_01225763	–	–	–	–
Fluvimarina pelagi str. HTCC 2506[c]	ZP_01439477; ZP_01439480	ZP_01439479; ZP_01439478	ZP_01439476	ZP_01439476; ZP_01439474	–	–	–	–
Phyllobacteriaceae								
Pseudoaminobacter salicylatoxidans str. KTC001	CAH59732; CAB94219[d]	CAH59733; CAC39169[d]	CAC39170[d]	CAH59734; CAH59735[d]	n.a.[d]	n.a.[d]	n.a.[d]	n.a.[d]
Bradyrhizobiaceae								
Bradyrhizobium japonicum str. USDA 110[c]	NP_770151/ NP_767654; NP_770154/ NP_767651/ NP_769372	NP_770152/ NP_769374; NP_770153/ NP_769373	NP_770155/ NP_767649	NP_770156/ NP_772761; NP_770157/ NP_772760	NP_773897; NP_773898	–	–	–
Bradyrizobium sp. str. BTAi1[c]	ZP_00857396/ ZP_00857569; ZP_00857393/ ZP_00863131	ZP_00857395/ ZP_00857570; ZP_00857394/ ZP_00857571	ZP_00857392/ ZP_00863133	ZP_00857391/ ZP_00862549; ZP_00857390/ ZP_00862550	–	–	–	–
Nitrobacter hamburgensis str. X14[c]	YP_578864; YP_578861	YP_578863; YP_578862	YP_578859	YP_576401; YP_576402	YP_578584; YP_578585	–	–	–
Nitrobacter sp. strain Nb-311A	–	–	–	–	ZP_01044876; ZP_01044877	–	–	–
Nitrobacter winogradskyi str. Nb-255	–	–	–	–	YP_319624; YP_319625	–	–	–
Rhodopseudomonas palustris str. BisA53	ZP_00810280; ZP_00810279	ZP_00810278; ZP_00810277	ZP_00810276	ZP_00810275; ZP_00810274	–	–	–	–
Rhodopseudomonas palustris str. BisB5	YP_571375; YP_571374	YP_571373; YP_571372	YP_571371	YP_571370; YP_571369	–	–	–	–
Rhodopseudomonas palustris str. BisB18	–	–	–	–	–	–	–	–
Rhodopseudomonas palustris str. HaA2	YP_487971; YP_487970	YP_487969; YP_487968	YP_487967	YP_487966; YP_487965	–	–	–	–
Rhodopseudomonas palustris str. CGA009	NP_949805; NP_949804	NP_949803; NP_949802	NP_949801	NP_949800; NP_949799	–	–	–	–
Hyphomicrobiaceae								
Starkeya novella	AAR98728; AAR98727[d]	AAR98726; AAR98725[d]	AAF61448[d]	AAF61449; AAF61450[d]	AAF64400; AAF64401[d]	n.a.[d]	n.a.[d]	n.a.[d]
Xanthobacter autotrophicus str. Py2	ZP_01196269; ZP_01196270	ZP_01196271; ZP_01196272	ZP_01196273	ZP_01196274; ZP_01196275	ZP_01199037/ ZP_01196335; ZP_01199083/ ZP_01196336	–	–	–

Table 4. *cont.*

	Homologues present in genome sequences of *Bacteria*[b]							
	Sox				Sor	Apr	Dsr	
Species[a]	SoxXA	SoxYZ	SoxB	SoxCD	SorAB	AprBA	DsrAB	DsrMKJOP
Proteobacteria phylum, *Betaproteobacteria*								
Burkholderiaceae								
Cupriavidus metallidurans str. CH34[c]	ZP_00593662; –	ZP_00593853; ZP_00593852	ZP_00593847	–	ZP_00595461; ZP_00595460	–	–	–
Polynucleobacter sp. str. QLW-P1DMWA-1[c]	ZP_01494652; ZP_01494653	ZP_01494655; ZP_01494654	ZP_01494651	ZP_01493496; ZP_01494656	ZP_01493045; ZP_01493143	–	–	–
Ralstonia eutrophica str. JMP134[c]	YP_297454; YP_297455	YP_297458; YP_297457	YP_297452	YP_297461; YP_297460	YP_297287; YP_297286	–	–	–
Ralstonia pickettii str. 12J[c]	ZP_01661485; ZP_01661484	ZP_01661481; ZP_01661482	ZP_01661487	–	YP_297287; YP_297286	–	–	–
Ralstonia solanacearum str. GMI1000[c]	NP_521374; NP_521375	NP_521378; NP_521377	NP_521372	–	NP_518934–3; NP_518932	–	–	–
Ralstonia solanacearum str. UW551[c]	ZP_00944484; ZP_00944483	ZP_00944482; ZP_00944481	ZP_00944480	–	ZP_00944736; ZP_00944735			
Comamonadaceae								
Comamonas testosteroni str. KF-1[c]	ZP_01521177; ZP_01521176	ZP_01521174; ZP_01521175	ZP_01521178	ZP_01521172; ZP_01521173	–	–	–	–
Polaromonas naphtalenivorans str. CJ2[c]	–	YP_981902; YP_981903	–	–	YP_982913; YP_982914	–	–	–
Polaromonas sp. str. JS666[c]	YP_549440; YP_549441	YP_549443; YP_549442	YP_549439	YP_549445; YP_549444	–	–	–	–
Oxalobacteraceae								
Herminiimonas arsenicoxydans str. KF-1[c]	CAL61371; CAL61370	CAL61368; CAL61369	CAL61372	CAL61365; CAL61376	CAL62480; CAL62479	–	–	–
Uncertain phylogenetic affiliation								
Methylibium petroleiphilum str. PM1[c]	YP_001021623; YP_001021624	YP_001021626; YP_001021625	YP_001021622	YP_001021628; YP_001021627	–	–	–	–
Hydrogenophilaceae								
Hydrogenophilus thermoluteolus str. TH-1	BAF34124; BAF34123[d]	BAF34121; BAF34122[d]	BAF34125[d]	BAF34119; BAF34120[d]	n.a.[d]	n.a.[d]	n.a.[d]	n.a.[d]
Thiobacillus denitrificans str. ATCC 25259	YP_314325/ YP_314675; YP_314322/ YP_314676	YP_314324; YP_314323	YP_314321	–	–	+	+	+
Rhodocyclaceae								
Dechloromonas aromatica str. RCB[c]	YP_286329; YP_286330	YP_286332; YP_286331	YP_286328	YP_286334; YP_286333	–	–	–	–
Proteobacteria phylum, *Gammaproteobacteria*								
Chromatiaceae								
Allochromatium vinosum str. DSM 180	ABE01360; ABE01361[d]	ABE01369; n.a.[d]	ABE01359[d]	n.a.[d]	n.a.[d]	+[d]	+[d]	+[d]

Ectothiorhodospiraceae								
Alkalimicola ehrlichei str. MHLE	–	YP_742517; YP_742518	–	–	–	–	+	+
Halorhodospira halophila str. SL1	YP_001003514; YP_001003514	YP_001003507; YP_001003508	YP_001003505	–	–	–	+	+
Piscirickettsiaceae								
Thiomicrospira crunogena str. XCL-2	YP_390874; YP_390871	YP_390873; YP_390872	YP_391815	YP_390426; YP_390427	–	–	–	–
Oceanospirillaceae								
Oceanospirillum sp. str. MED92[c]	ZP_01167154; ZP_01167150	ZP_01167153; ZP_01167151	ZP_01167148	ZP_01167156; ZP_01167155	–	–	–	–
Uncertain phylogenetic affiliation								
‘*Candidatus* Ruthia magnifica’ str. CM[c]	YP_904000; YP_903997	YP_903999; YP_903998	YP_903419	–	–	+	+	+
Endoriftia persephone[c]	+ +[e]	–; +[e]	+[e]	–[e]	–[e]	+[e]	+[e]	+[e]
Olavius algarvensis Gamma-1 symbiont[c]	–; –[e]	+ +[e]	+[e]	–[e]	–[e]	+[e]	+[e]	+[e]
Olavius algarvensis Gamma-3 symbiont[c]	–; –[e]	+ +[e]	+[e]	–[e]	–[e]	+[e]	+[e]	+[e]
Congregibacter litoralis str. KT 71[c]	ZP_01102561; ZP_01102558	ZP_01102560; ZP_01102559	ZP_01102556	ZP_01102563; ZP_01102562	–	–	–	–
Marine gammaproteobacterium str. HTCC 2080[c]	ZP_01627096; ZP_01627097	ZP_01627099; ZP_01627098	ZP_01627095	ZP_01627101; ZP_01627100	–	–	–	–
Proteobacteria phylum, *Deltaproteobacteria*								
Cystobacteraceae								
Anaeromyxobacter dehalogenans str. 2CP-C[c]	YP_465487; YP_465488	YP_465491; YP_465490	YP_465486	YP_465493; YP_465492	–	–	–	–
Proteobacteria phylum, *Epsilonproteobacteria*								
Helicobacteraceae								
Sulfurimonas denitrificans str. ATCC 33889	YP_392776; YP_392779	YP_394567/ YP_392777; YP_392778	YP_392780	YP_394569/ YP_394568	–	–	–	–
Unclassified *Proteobacteria*								
Magnetococcus sp. str. MC-1[c]	YP_867608/ YP_865823; YP_867605/ YP_865820	YP_867607/ YP_865822; YP_867606/ YP_866895	YP_865819	–	–	–	–	–

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a. Taxonomic classification according to the taxonomic outline of the prokaryotes, Bergey’s Manual of Systematic Bacteriology, 2nd edition, release 5.0 May 2004 (http://dx.doi.org/10.1007/bergeysoutline).

b. Abbreviations: (n.a.) sequence information for respective protein encoding gene is not available (no genome sequencing project of respective species); (–) no protein encoding homologue identified by BLAST search in genome; (+) protein encoding homologue identified by BLAST search in genome.

c. Thiosulfate-oxidizing ability of respective species not experimentally proven.

d. No genome sequencing project: sequence information of Sox, Sor, Apr or Dsr encoding homologues retrieved from cloning experiments of the respective species.

e. No genome sequencing project: sequence information of Sox, Sor, Apr or Dsr encoding homologues retrieved from metagenomic sequencing project of the respective species.

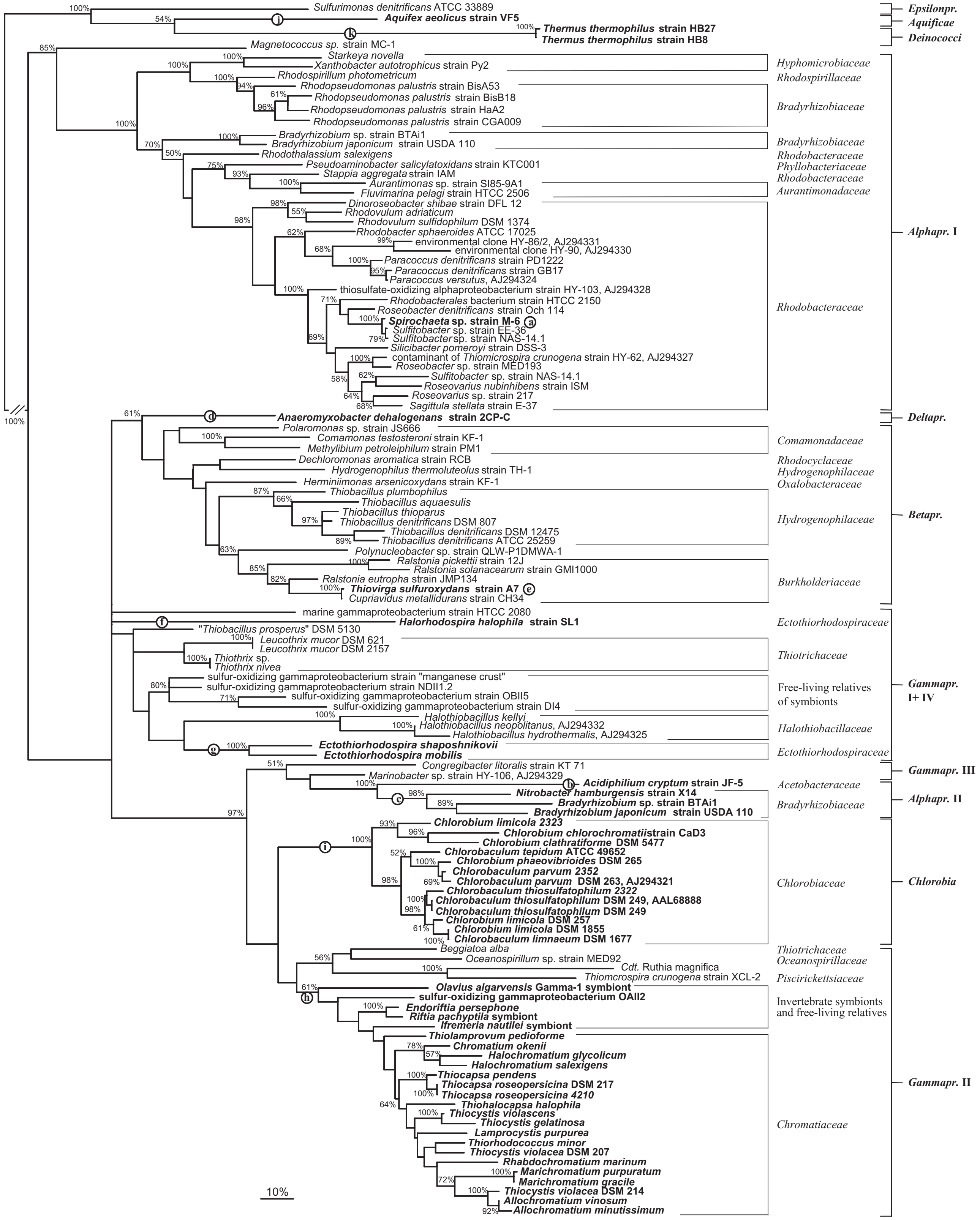
Sulfurimonas denitrificans ATCC 33889
Aquifex aeolicus strain VF5
Thermus thermophilus strain HB27
Thermus thermophilus strain HB8
Magnetococcus sp. strain MC-1
Starkeya novella
Xanthobacter autotrophicus strain Py2
Rhodospirillum photometricum
Rhodopseudomonas palustris strain BisA53
Rhodopseudomonas palustris strain BisB18
Rhodopseudomonas palustris strain HaA2
Rhodopseudomonas palustris strain CGA009
Bradyrhizobium sp. strain BTAi1
Bradyrhizobium japonicum strain USDA 110
Rhodothalassium salexigens
Pseudoaminobacter salicylatoxidans strain KTC001
Stappia aggregata strain IAM
Aurantimonas sp. strain SI85-9A1
Fluvimarina pelagi strain HTCC 2506
Dinoroseobacter shibae strain DFL 12
Rhodovulum adriaticum
Rhodovulum sulfidophilum DSM 1374
Rhodobacter sphaeroides ATCC 17025
environmental clone HY-86/2, AJ294331
environmental clone HY-90, AJ294330
Paracoccus denitrificans strain PD1222
Paracoccus denitrificans strain GB17
Paracoccus versutus, AJ294324
thiosulfate-oxidizing alphaproteobacterium strain HY-103, AJ294328
Rhodobacterales bacterium strain HTCC 2150
Roseobacter denitrificans strain Och 114
Spirochaeta sp. strain M-6 ⓐ
Sulfitobacter sp. strain EE-36
Sulfitobacter sp. strain NAS-14.1
Silicibacter pomeroyi strain DSS-3
contaminant of Thiomicrospira crunogena strain HY-62, AJ294327
Roseobacter sp. strain MED193
Sulfitobacter sp. strain NAS-14.1
Roseovarius nubinhibens strain ISM
Roseovarius sp. strain 217
Sagittula stellata strain E-37
Anaeromyxobacter dehalogenans strain 2CP-C
Polaromonas sp. strain JS666
Comamonas testosteroni strain KF-1
Methylibium petroleiphilum strain PM1
Dechloromonas aromatica strain RCB
Hydrogenophilus thermoluteolus strain TH-1
Herminiimonas arsenicoxydans strain KF-1
Thiobacillus plumbophilus
Thiobacillus aquaesulis
Thiobacillus thioparus
Thiobacillus denitrificans DSM 807
Thiobacillus denitrificans DSM 12475
Thiobacillus denitrificans ATCC 25259
Polynucleobacter sp. strain QLW-P1DMWA-1
Ralstonia pickettii strain 12J
Ralstonia solanacearum strain GMI1000
Ralstonia eutropha strain JMP134
Thiovirga sulfuroxydans strain A7 ⓔ
Cupriavidus metallidurans strain CH34
marine gammaproteobacterium strain HTCC 2080
Halorhodospira halophila strain SL1
"Thiobacillus prosperus" DSM 5130
Leucothrix mucor DSM 621
Leucothrix mucor DSM 2157
Thiothrix sp.
Thiothrix nivea
sulfur-oxidizing gammaproteobacterium strain "manganese crust"
sulfur-oxidizing gammaproteobacterium strain NDII1.2
sulfur-oxidizing gammaproteobacterium strain OBII5
sulfur-oxidizing gammaproteobacterium strain DI4
Halothiobacillus kellyi
Halothiobacillus neopolitanus, AJ294332
Halothiobacillus hydrothermalis, AJ294325
Ectothiorhodospira shaposhnikovii
Ectothiorhodospira mobilis
Congregibacter litoralis strain KT 71
Marinobacter sp. strain HY-106, AJ294329
Acidiphilium cryptum strain JF-5
Nitrobacter hamburgensis strain X14
Bradyrhizobium sp. strain BTAi1
Bradyrhizobium japonicum strain USDA 110
Chlorobium limicola 2323
Chlorobium chlorochromatii strain CaD3
Chlorobium clathratiforme DSM 5477
Chlorobaculum tepidum ATCC 49652
Chlorobium phaeovibrioides DSM 265
Chlorobaculum parvum 2352
Chlorobaculum parvum DSM 263, AJ294321
Chlorobaculum thiosulfatophilum 2322
Chlorobaculum thiosulfatophilum DSM 249, AAL68888
Chlorobaculum thiosulfatophilum DSM 249
Chlorobium limicola DSM 257
Chlorobium limicola DSM 1855
Chlorobaculum limnaeum DSM 1677
Beggiatoa alba
Oceanospirillum sp. strain MED92
Cdt. Ruthia magnifica
Thiomcrospira crunogena strain XCL-2
Olavius algarvensis Gamma-1 symbiont
sulfur-oxidizing gammaproteobacterium OAII2
Endoriftia persephone
Riftia pachyptila symbiont
Ifremeria nautilei symbiont
Thiolamprovum pedioforme
Chromatium okenii
Halochromatium glycolicum
Halochromatium salexigens
Thiocapsa pendens
Thiocapsa roseopersicina DSM 217
Thiocapsa roseopersicina 4210
Thiohalocapsa halophila
Thiocystis violascens
Thiocystis gelatinosa
Lamprocystis purpurea
Thiorhodococcus minor
Thiocystis violacea DSM 207
Rhabdochromatium marinum
Marichromatium purpuratum
Marichromatium gracile
Thiocystis violacea DSM 214
Allochromatium vinosum
Allochromatium minutissimum
ⓑ ⓒ ⓓ ⓕ ⓖ ⓗ ⓘ ⓙ ⓚ
10%
Hyphomicrobiaceae
Rhodospirillaceae
Bradyrhizobiaceae
Bradyrhizobiaceae
Rhodobacteraceae
Phyllobacteriaceae
Rhodobacteraceae
Aurantimonadaceae
Rhodobacteraceae
Comamonadaceae
Rhodocyclaceae
Hydrogenophilaceae
Oxalobacteraceae
Hydrogenophilaceae
Burkholderiaceae
Ectothiorhodospiraceae
Thiotrichaceae
Free-living relatives of symbionts
Halothiobacillaceae
Ectothiorhodospiraceae
Acetobacteraceae
Bradyrhizobiaceae
Chlorobiaceae
Thiotrichaceae
Oceanospirillaceae
Piscirickettsiaceae
Invertebrate symbionts and free-living relatives
Chromatiaceae
Epsilonpr.
Aquificae
Deinococci
Alphapr. I
Deltapr.
Betapr.
Gammapr. I+ IV
Gammapr. III
Alphapr. II
Chlorobia
Gammapr. II

Fig. 1. SoxB consensus tree based on 124 SoxB sequences from the investigated SOB including the full-length SoxB sequences retrieved from the public databases. Polytomic nodes connect branches for which a relative order could not be determined unambiguously by applying distance matrix-based, maximum parsimony and maximum likelihood methods. Maximum likelihood bootstrap re-sampling values greater than 50% (100 re-samplings) are indicated near the nodes. The SoxB sequences of *Sulfurimonas denitrificans*, *Aquifex aeolicus* and *Thermus thermophilus* ssp. were used as outgroup references. Sulfur-oxidizing bacteria (SOB) with putative laterally transferred *soxB* genes are in boldface; proposed LGT events are indicated by letters (a–k). The 16S rRNA gene-based taxonomical classification of SOB species is indicated. The scale bar corresponds to 10% estimated sequence divergence.

and *Chromatiaceae* revealed that the 16S rRNA gene-based species relationships are not reflected in the SoxB tree topology. Interestingly, the latter is consistent to the AprBA-based tree topology (B. Meyer and J. Kuever, 2007b); both protein phylogenies point to an incorrect classification of SOB strain DSM 214 as *Thiocystis violacea* subspecies (see also Fig. 2). The 16S rRNA gene-discordant affiliation of the epsilonproteobacterial *S. denitrificans* (Takai *et al*., 2006) with the hyperthermophilic *Aquifex aeolicus* and *Thermus thermophilus* ssp. near the root of the SoxB tree indicates their involvement in LGT events (LGTs j and k).

Additional evidence for lateral transfer of soxB *genes*

Additional evidence for the inferred phylogenetic position of the SOB taxa in the SoxB tree is given by the presence of insertions and deletions (indels) at identical sequence positions (see Table S1). The comparison of the aligned SoxB sequences supports the distinct branching position from *S. denitrificans* and representatives of the *Aquificae* and *Thermaceae* by the presence of several unique indels. The xenology of the SoxB from *Spirochaeta* sp. strain M-6, *T. sulfuroxydans* strain A7, *A. dehalogenans* and members of *Alphaproteobacteria* II is confirmed by the presence of *Roseobacter*-, *Betaproteobacteria*- and *Gammaproteobacteria* cluster III-specific indels respectively. In addition, the 16S rRNA gene-discordant affiliations of the anaerobic anoxygenic phototrophic SOB lineages with the *Gammaproteobacteria* clusters I to III are supported by shared, distinctive indels, while the separate branching position of *H. halophila* (cluster IV) is confirmed by *Beta*- and *Gammaproteobacteria* cluster I-specific as well as two unique indels.

Atypical sequence characteristics, e.g. significant deviations in G + C content and codon usage between the proposed LGT-derived *soxB* gene and the recipient genome, are useful as signposts for recent events of LGT. In general, no indications for recent LGT events were identified among the presumed LGT-affected SOB with the exception of the *T. sulfuroxydans* strain A7. This strain has a genome G + C content of 47.1%, while its *soxB* G + C content (64.2%) and codon usage are nearly identical to those of the putative donor strain *Cupriavidus metallidurans* strain CH34 (G + C content of *soxB* and genome, 65.9% and 63.7%, respectively).

Correlation between the sox *gene cluster composition and the occurrence of* dsr *genes in genomes of sulfur-storing SOB*

Genome data concerning the *sox* gene cluster, *soxXAYZBCD*, were available from 61 different *Proteobacteria* and *Chlorobiaceae* species, *A. aeolicus* and two *T. thermophilus* strains. The comparison of the genomic gene content revealed that the presence of the *dsrAB*/*dsrMKJOP* correlated with the absence of *soxCD* genes: all thiosulfate-oxidizing species that are known to intermediately deposit elemental sulfur lack the sulfur dehydrogenase encoding genes of the periplasmic Sox enzyme system but possess the genetic ability to oxidize the stored sulfur via the cytoplasmic dissimilatory sulfite reductase (DsrAB), e.g. (i) the *Chlorobiaceae,* (ii) *Allochromatium vinosum* and *H. halophila* (as representatives of the *Chromatiaceae* and *Ectothiorhodospiraceae*, respectively), (iii) *Thiobacillus denitrificans*, and (iv) ‘*Cdt.* R. magnifica’. In contrast, the majority of *sox* gene-containing *Alpha*-, *Beta*- and *Gammaproteobacteria*, *S. denitrificans* and *T. thermophilus* ssp. harboured a complete, *Paracoccus pantotrophus-/Rhodovulum sulfidophilum*-homologous *sox* gene cluster (Appia-Ayme *et al*., 2001; Friedrich *et al*., 2001) in their genomes and lacked the *dsrAB*/*dsrMKJOP* genes. Notably, the presence of the *sox* gene cluster differed at the species (*Chlorobium*, *Silicibacter*, *Nitrobacter* and *Polaromonas*) and subspecies (*Rhodobacter sphaeroides* and *Rhodopseudomonas palustris*) level.

Discussion

Distribution of soxB *genes among photo- and chemotrophic SOB*

The members of the anaerobic anoxygenic phototrophic *Chlorobiaceae*, *Chromatiaceae* and *Ectothiorhodospiraceae* and aerobic chemotrophic *Beggiatoa*, *Thiothrix*, *Thiobacillus*, *Thiomicrospira* and free-living relatives of invertebrate symbionts form intra- and extracellularly stored sulfur globules as obligate intermediate during thiosulfate oxidation (Nelson and Fisher, 1995; Howarth *et al*., 1999; Imhoff, 1999; 2001a; 2003; Kuever *et al*., 2002; Robertson and Kuenen, 2002; Teske and Nelson, 2004). Based on recent experimental results on sulfur-storing *Chlorobaculum tepidum* (Hanson and Tabita, 2003),

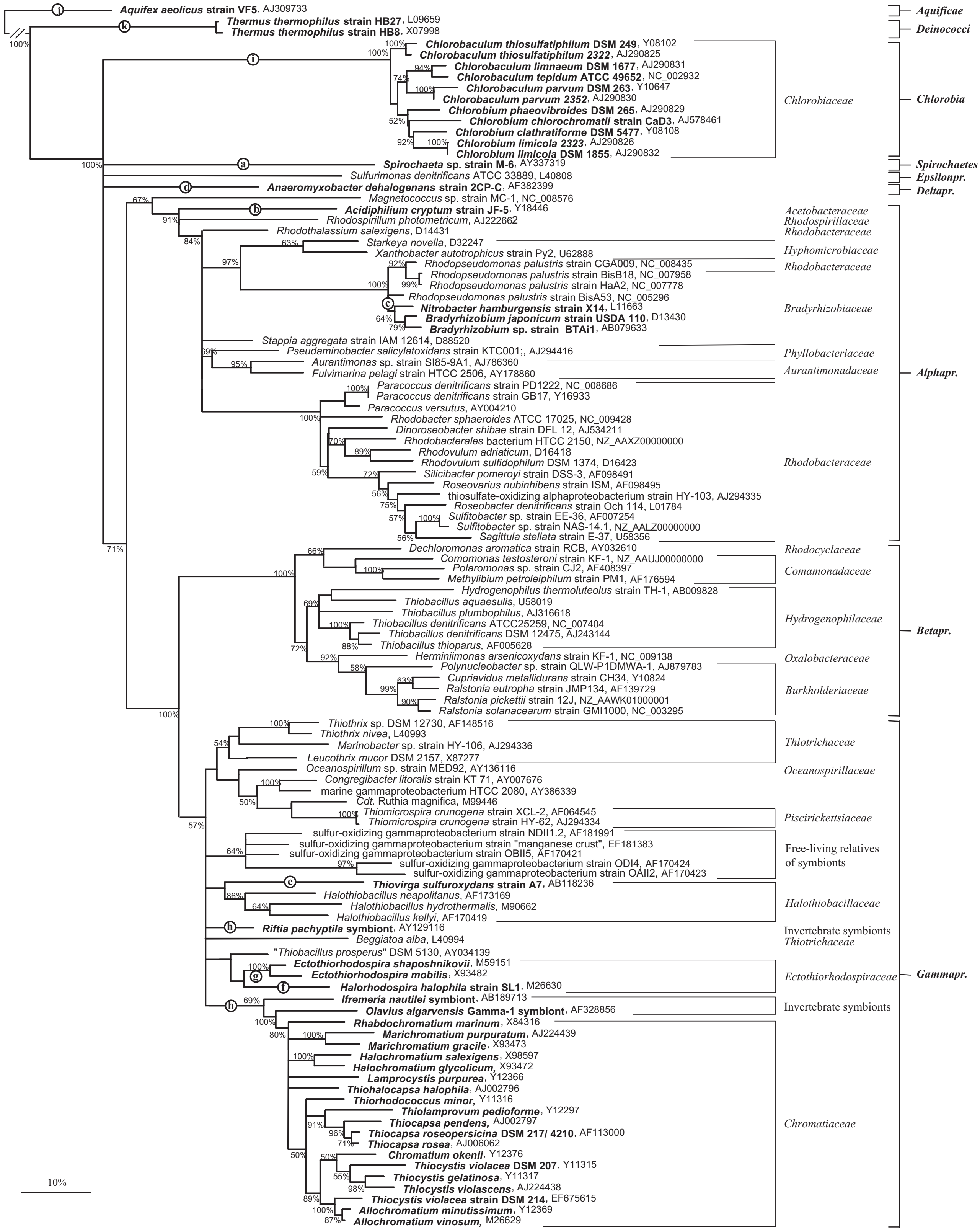
j
Aquifex aeolicus strain VF5, AJ309733
k
Thermus thermophilus strain HB27, L09659
Thermus thermophilus strain HB8, X07998
100%
i
Chlorobaculum thiosulfatiphilum DSM 249, Y08102
Chlorobaculum thiosulfatiphilum 2322, AJ290825
Chlorobaculum limnaeum DSM 1677, AJ290831
Chlorobaculum tepidum ATCC 49652, NC_002932
Chlorobaculum parvum DSM 263, Y10647
Chlorobaculum parvum 2352, AJ290830
Chlorobium phaeovibroides DSM 265, AJ290829
Chlorobium chlorochromatii strain CaD3, AJ578461
Chlorobium clathratiforme DSM 5477, Y08108
Chlorobium limicola 2323, AJ290826
Chlorobium limicola DSM 1855, AJ290832
a
Spirochaeta sp. strain M-6, AY337319
Sulfurimonas denitrificans ATCC 33889, L40808
d
Anaeromyxobacter dehalogenans strain 2CP-C, AF382399
Magnetococcus sp. strain MC-1, NC_008576
b
Acidiphilium cryptum strain JF-5, Y18446
Rhodospirillum photometricum, AJ222662
Rhodothalassium salexigens, D14431
Starkeya novella, D32247
Xanthobacter autotrophicus strain Py2, U62888
Rhodopseudomonas palustris strain CGA009, NC_008435
Rhodopseudomonas palustris strain BisB18, NC_007958
Rhodopseudomonas palustris strain HaA2, NC_007778
Rhodopseudomonas palustris strain BisA53, NC_005296
c
Nitrobacter hamburgensis strain X14, L11663
Bradyrhizobium japonicum strain USDA 110, D13430
Bradyrhizobium sp. strain BTAi1, AB079633
Stappia aggregata strain IAM 12614, D88520
Pseudaminobacter salicylatoxidans strain KTC001;, AJ294416
Aurantimonas sp. strain SI85-9A1, AJ786360
Fulvimarina pelagi strain HTCC 2506, AY178860
Paracoccus denitrificans strain PD1222, NC_008686
Paracoccus denitrificans strain GB17, Y16933
Paracoccus versutus, AY004210
Rhodobacter sphaeroides ATCC 17025, NC_009428
Dinoroseobacter shibae strain DFL 12, AJ534211
Rhodobacterales bacterium HTCC 2150, NZ_AAXZ00000000
Rhodovulum adriaticum, D16418
Rhodovulum sulfidophilum DSM 1374, D16423
Silicibacter pomeroyi strain DSS-3, AF098491
Roseovarius nubinhibens strain ISM, AF098495
thiosulfate-oxidizing alphaproteobacterium strain HY-103, AJ294335
Roseobacter denitrificans strain Och 114, L01784
Sulfitobacter sp. strain EE-36, AF007254
Sulfitobacter sp. strain NAS-14.1, NZ_AALZ00000000
Sagittula stellata strain E-37, U58356
Dechloromonas aromatica strain RCB, AY032610
Comomonas testosteroni strain KF-1, NZ_AAUJ00000000
Polaromonas sp. strain CJ2, AF408397
Methylibium petroleiphilum strain PM1, AF176594
Hydrogenophilus thermoluteolus strain TH-1, AB009828
Thiobacillus aquaesulis, U58019
Thiobacillus plumbophilus, AJ316618
Thiobacillus denitrificans ATCC25259, NC_007404
Thiobacillus denitrificans DSM 12475, AJ243144
Thiobacillus thioparus, AF005628
Herminiimonas arsenicoxydans strain KF-1, NC_009138
Polynucleobacter sp. strain QLW-P1DMWA-1, AJ879783
Cupriavidus metallidurans strain CH34, Y10824
Ralstonia eutropha strain JMP134, AF139729
Ralstonia pickettii strain 12J, NZ_AAWK01000001
Ralstonia solanacearum strain GMI1000, NC_003295
Thiothrix sp. DSM 12730, AF148516
Thiothrix nivea, L40993
Marinobacter sp. strain HY-106, AJ294336
Leucothrix mucor DSM 2157, X87277
Oceanospirillum sp. strain MED92, AY136116
Congregibacter litoralis strain KT 71, AY007676
marine gammaproteobacterium HTCC 2080, AY386339
Cdt. Ruthia magnifica, M99446
Thiomicrospira crunogena strain XCL-2, AF064545
Thiomicrospira crunogena strain HY-62, AJ294334
sulfur-oxidizing gammaproteobacterium strain NDII1.2, AF181991
sulfur-oxidizing gammaproteobacterium strain "manganese crust", EF181383
sulfur-oxidizing gammaproteobacterium strain OBII5, AF170421
sulfur-oxidizing gammaproteobacterium strain ODI4, AF170424
sulfur-oxidizing gammaproteobacterium strain OAII2, AF170423
e
Thiovirga sulfuroxydans strain A7, AB118236
Halothiobacillus neapolitanus, AF173169
Halothiobacillus hydrothermalis, M90662
Halothiobacillus kellyi, AF170419
h
Riftia pachyptila symbiont, AY129116
Beggiatoa alba, L40994
"Thiobacillus prosperus" DSM 5130, AY034139
Ectothiorhodospira shaposhnikovii, M59151
g
Ectothiorhodospira mobilis, X93482
f
Halorhodospira halophila strain SL1, M26630
h
Ifremeria nautilei symbiont, AB189713
Olavius algarvensis Gamma-1 symbiont, AF328856
Rhabdochromatium marinum, X84316
Marichromatium purpuratum, AJ224439
Marichromatium gracile, X93473
Halochromatium salexigens, X98597
Halochromatium glycolicum, X93472
Lamprocystis purpurea, Y12366
Thiohalocapsa halophila, AJ002796
Thiorhodococcus minor, Y11316
Thiolamprovum pedioforme, Y12297
Thiocapsa pendens, AJ002797
Thiocapsa roseopersicina DSM 217/ 4210, AF113000
Thiocapsa rosea, AJ006062
Chromatium okenii, Y12376
Thiocystis violacea DSM 207, Y11315
Thiocystis gelatinosa, Y11317
Thiocystis violascens, AJ224438
Thiocystis violacea strain DSM 214, EF675615
Allochromatium minutissimum, Y12369
Allochromatium vinosum, M26629
Chlorobiaceae
Acetobacteraceae
Rhodospirillaceae
Rhodobacteraceae
Hyphomicrobiaceae
Rhodobacteraceae
Bradyrhizobiaceae
Phyllobacteriaceae
Aurantimonadaceae
Rhodobacteraceae
Rhodocyclaceae
Comamonadaceae
Hydrogenophilaceae
Oxalobacteraceae
Burkholderiaceae
Thiotrichaceae
Oceanospirillaceae
Piscirickettsiaceae
Free-living relatives of symbionts
Halothiobacillaceae
Invertebrate symbionts
Thiotrichaceae
Ectothiorhodospiraceae
Invertebrate symbionts
Chromatiaceae
Aquificae
Deinococci
Chlorobia
Spirochaetes
Epsilonpr.
Deltapr.
Alphapr.
Betapr.
Gammapr.
10%

Fig. 2. Consensus tree based on the 16S rRNA gene sequences of the *soxB* gene-containing SOB species as indicated by the genetic and genomic analyses of this study. Polytomic nodes connect branches for which a relative order could not be determined unambiguously by applying distance matrix-based, maximum parsimony and maximum likelihood methods. Maximum likelihood bootstrap re-sampling values greater than 50% (100 re-samplings) are indicated near the nodes. The 16S rRNA gene sequence of *Aquifex aeolicus* was used as an outgroup reference. Sulfur-oxidizing bacteria (SOB) with putative laterally transferred *soxB* genes are in boldface; proposed LGT events are indicated by letters (a–k, see Fig. 1). The scale bar corresponds to 10% estimated sequence divergence.

A. vinosum (Hensen *et al*., 2006) and *T. denitrificans* (Beller *et al*. 2006), the truncated Sox enzyme system, SoxXAYZB, was postulated to be functionally linked to the reverse-acting enzymes of the cytoplasmic sulfate-reduction pathway (Friedrich *et al*., 2005; Hensen *et al*., 2006): in analogy to the *P. pantotrophus*-based mechanism (Friedrich *et al*., 2001), the SoxXA would oxidatively couple thiosulfate to a cysteine-sulfhydryl group of the SoxYZ complex from which sulfate would be hydrolysed off by SoxB. Due to the lack of the sulfur dehydrogenase $Sox(CD)_2$ component, the sulfane sulfur of thiosulfate would be transferred to the sulfur globules and subsequently oxidized to sulfate via the reverse dissimilatory sulfite reductase, APS reductase, ATP sulfurylase and sulfite:acceptor oxidoreductase. Indeed, the previous proteins have been identified in several members of the anaerobic anoxygenic phototrophic SOB lineages as well as chemolithotrophic *T. denitrificans*, marine *Beggiatoa*, invertebrate symbionts and their free-living relatives (Brune, 1995; Nelson and Fisher, 1995; Pott and Dahl, 1998; Dahl *et al*., 1999; 2005; Kappler and Dahl, 2001; Sanchez *et al*., 2001; Kuever *et al*., 2002; Teske and Nelson, 2004), whereas the general presence of Sox proteins was unconfirmed for most sulfur-storing species. The present study confirmed the ubiquitous presence of the *soxB* gene in all known thiosulfate-oxidizing, sulfur-storing chemo- and phototrophic SOB species but also for species that have not yet been reported to use this sulfur compounds as electron donor (e.g. *C. limicola* DSM 1855, *Thiocystis gelatinosa*, *Ectothiorhodospira marismortui*, *Leucothrix mucor*, *Spirochaeta* sp.) (see Table 1). As the *soxB* is generally a part of the *sox* gene cluster (see Table 4), its PCR-based detection in the respective SOB species might be used as a first indication for the putative presence of components of the Sox enzyme system. In context with the absence of *soxCD* genes and the presence of genes coding for the reverse dissimilatory sulfate-reduction pathway in the accessible genomes of *Chlorobiaceae*, *A. vinosum*, *H. halophila*, *T. denitrificans* and '*Cdt.* R. magnifica' (Table 4), the recently postulated model for a general involvement of the Sox enzyme system in the thiosulfate oxidation in sulfur-storing bacteria is therefore supported by the results of our study (Friedrich *et al*., 2005; Hensen *et al*., 2006).

The PCR amplification results are most likely false-negative for the examined *Thiomicrospira* spp. and related symbionts of Mytilid mussels as well as Vesicomyid clams as *T. crunogena* and '*Cdt.* R. magnifica' harbour *soxB* genes with non-complementary primer target sites. Indeed, the investigated *Thiomicrospira* spp. have been demonstrated to oxidize thiosulfate to sulfate (Brinkhoff *et al*., 1999) (note: *T. crunogena* deposits sulfur globules despite the presence of a *P. pantotrophus*-homologous *sox* gene cluster and the absence of *dsr* and *apr* genes). In contrast, the thiosulfate-oxidizing abilities of the symbiotic bacteria have not been investigated in detail (Nelson and Fisher, 1995; Cavanaugh *et al*., 2004). The *soxB* target site of *Endoriftia persephone* and *Olavius algarvensis* Gamma-1/-3 symbionts are complementary to the primers used in the PCR assays; thus, the absence of the *soxB* in certain symbiotic bacteria might be correct and reflect the preferred utilization of sulfide as energy source, as it is generally proposed for invertebrate symbionts (Cavanaugh *et al*., 2004). Direct supply of thiosulfate to their symbionts has only been reported for *Bathymodiolus thermophilus* and *Calyptogena magnifica* that detoxify sulfide by conversion to this less reduced sulfur compound (Nelson and Fisher, 1995; Cavanaugh *et al*., 2004).

In support of the postulated wide distribution of the Sox enzyme system-mediated pathway as a common mechanism for bacterial thiosulfate oxidation (Friedrich *et al*., 2001; 2005), the collected genomic data demonstrated the complete *sox* gene cluster to be present in various photo- and chemotrophic representatives of the *Proteobacteria* as well as hyperthermophilic *T. thermophilus* ssp.; however, for most of these species the ability to utilize thiosulfate has not been experimentally confirmed (see Table 4), and thus the presence of an operative, *P. pantotrophus*-/*R. sulfidophilum*-homologous Sox enzyme system is speculative until experimentally proven. Nevertheless, the abundance of *sox* genes in aerobic photo- and non-phototrophic species of the marine *Roseobacter* clade points to the energetical benefit of the Sox enzyme system-mediated oxidation of inorganic sulfur compounds for members of the latter group that generally dominate the degradation of organic sulfur compounds in the bacterioplankton community (Buchan *et al*., 2005). In contrast, the capability to use reduced inorganic sulfur compounds as photosynthetic electron donors is restricted among anaerobic anoxygenic phototrophic members of the *Alphaproteobacteria* to certain genera (Brune, 1995; Imhoff, 2001b). This is reflected by the limited detection of the *soxB* gene in *Rhodothalassium*, *Rhodospirillum* and *Rhodovulum* species in the PCR assays of this study (Table 1), although false-negative amplification results cannot be completely ruled out, e.g. for thiosulfate-oxidizing *R. acidophilus* as *R. palustris*-relative (Imhoff, 2001b).

In SOB living in extreme habitats, such as *Acidithiobacillus*, *Halothiobacillus* and *Thermothiobacillus*, the complete oxidation of thiosulfate to sulfate has been suggested to be performed via polythionates (Pronk *et al*., 1990; Meulenberg *et al*., 1993; Kelly *et al*., 1997). For acidophiles such a pathway makes perfect sense, allowing rapid conversion of thiosulfate, which is chemically unstable under acidic conditions, into an acid-stable intermediate (tetrathionate). Interestingly, the *soxB* gene was identified in the acidophilic '*T. prosperus'* (Huber and Stetter, 1989), which might be a first indication that the Sox enzyme system is also present in some acidophilic SOB; however, further experimental investigation is needed for verification (note: *Acidithiobacillus ferrooxidans* harbours no *sox* homologues in its genome). The ability to use more than one thiosulfate-oxidizing enzymatic system/enzyme, e.g. the incomplete Sox system plus Dsr and a thiosulfate dehydrogenase as reported for *A. vinosum* (Hensen *et al*., 2006), allows an adaptation of the energy conservation to the varying physico-chemical conditions in environment.

Phylogeny of SoxB: evidence for LGT among SOB

Multiple events of lateral *soxB* gene transfer among the SOB are the most reasonable explanation for (i) the inferred close relationships of SoxB from SOB species that are distantly related on the basis of the 16S rRNA gene phylogeny, e.g. *S. denitrificans*, *A. aeolicus* and *T. thermophilus* ssp., and (ii) the presence of two distantly related *soxB* genes in the genome of the same organism, e.g. *Bradyrhizobium* species (Figs 1 and 2). The betaproteobacterial and the gammaproteobacterial strains that served as donors for the LGT-affected *Bradyrhizobiaceae, Acetobacteraceae* (*Alphaproteobacteria* lineage II) and *A. dehalogenans* respectively, are not apparent. The *Bradyrhizobium* spp. and related *N. hamburgensis* strain X14 might have acquired their *soxB* gene by independent LGT events. Alternatively, a single LGT might have affected their ancestor prior to the diversification of *Bradyrhizobium* and *Nitrobacter*, which was followed by a replacement of the authentic *soxB* gene by the xenolog in the ancestor of *Nitrobacter* (the xenolog will have later been lost by most *Nitrobacter* spp. except *N. hamburgensis* strain X14, see Table 4). The high sequence identity values of the partial SoxB sequences from *Spirochaeta* sp. strain M-6 and *T. sulfuroxydans* strain A7 to those of their putative donor strains, *Sulfitobacter* and *Ralstonia* spp. (98.3% and 99.5%, respectively), are indicative for recent lateral transfers. However, genome data of *Spirochaeta* sp. strain M-6 are needed for verification. The coexistence of recipient and potential donor strains have been reported, e.g. in '*Thiodendron*' sulfur bacterial mats and sulfur-containing microaerobic wastewaters and sludge (Qureshi *et al*., 2003; Dubinina *et al*., 2004; Ito *et al*., 2004) that would have enabled interspecies gene exchange.

According to the SoxB tree, the *Gammaproteobacteria* are not monophyletic. The anaerobic anoxygenic phototrophic lineages are 16S rRNA-discordantly affiliated to the different chemotrophic SOB lineages (*Gammaproteobacteria* I or II). Therefore, the genera of the *Ectothiorhodospiraceae* (*Ectothio*- and *Halorhodospira*) and the *Chromatiaceae* (and affiliated invertebrate symbionts), as well as the *Chlorobiaceae*, are proposed to have received their *soxB* genes by four independent LGT events with different chemotrophic SOB of the *Gammaproteobacteria* having served as donors, e.g. moderate halophilic *Ectothiorhodospiraceae* and habitat-sharing Halothiobacilli (Imhoff, 1999; Kelly and Wood, 2000). These transfers most likely occurred before their diversification, which was followed by a *sox* gene loss in those genera that are described as metabolically less versatile, e.g. *Thiococcus* and *Prosthecochloris* spp. (Imhoff, 1999; 2001a; 2003). All proteobacterial SoxB lineages comprise chemotrophic SOB with *P. pantotrophus*-/*R. sulfidophilum*-homologous *sox* gene clusters in their genomes, whereas the xenologous anaerobic anoxygenic phototrophic SOB lineages (including invertebrate symbionts) harbour truncated gene loci. This might indicate that initially the ancestors of the latter groups acquired the complete *soxXAYZBCD* gene cluster from their chemotrophic donors [note: the *sox* gene cluster is located on a endogenous plasmid in certain green sulfur bacteria, and its successful lateral transfer to non-thiosulfate-utilizing strains was demonstrated (Mendez-Alvarez *et al*., 1994)]. In adaptation, the Sox enzyme pathway could have been functionally linked to the pre-existing cytoplasmic sulfide/elemental sulfur oxidation pathway (DsrAB/DsrMKJOP) and the *soxCD* genes were subsequently lost, which resulted in the recognized thiosulfate oxidation pathway via sulfur-globule formation. Alternatively, this process could have happened in the potential sulfur-storing chemotrophic donors of *Chromatiaceae* and *Chlorobiaceae* prior to the LGTs.

With regard to the 16S rRNA gene-discordant relationship of *S. denitrificans*, *A. aeolicus* and *T. thermophilus* ssp. at the root of the SoxB tree, there are two possible scenarios for the direction of LGT and the origin of the SoxB protein. First, if the *soxB* of the hyperthermophilic species is assumed to be xenologous, a (epsilon-)proteobacterial origin of the SoxB protein would be consistent with the tree topology. In support, all currently available sequences of other non-proteobacterial SOB species (*Chlorobiaceae*, *Spirochaeta* sp. strain M-6) seem to be laterally acquired from *Proteobacteria*. Indeed, recent phylogenomic studies disputed the 16S rRNA gene-based basal branching of *Aquifex* but placed it next to the *Epsilon-/Deltaproteobacteria* (Dutilh *et al*. 2004). Second, if the SoxB of *S. denitrificans* is assumed to be xenologous, the tree topology would indicate a *soxB* origin within the *Aquificales* or the *Thermus* lineage followed by a LGT to the evolving proteobacterial lineages. Irrespective of scenario, exchange of genetic material between these phylogenetic groups would have been possible, as various molecular studies confirmed their coexistence and dominance at hydrothermal vents (Reysenbach *et al*., 2000; Takai *et al*., 2005; Campbell *et al*., 2006).

Potential evolutionary scenario for the Sox enzyme pathway in SOB

During the Proterozoic era, the ocean was proposed to have been globally anoxic and sulfidic (Shen *et al*., 2003; Canfield, 2005) with a widespread occurrence and predominance of planktonic ancestors of the *Chromatiaceae* and *Chlorobiaceae* lineages as demonstrated by molecular fossils (Brocks *et al*., 2005). The anoxic formation of thiosulfate via (i) chemical FeS_2 oxidation with MnO_2 and (ii) biogenic FeS oxidation by denitrifying bacteria (Schippers, 2004) would have been absent. As the dissimilatory sulfite and APS reductase phylogenies point to an ancient origin of the sulfate reduction/sulfide oxidation pathway in SRP and SOB (Boucher *et al*., 2003; Meyer and Kuever, 2007a) as early as 3.47 giga annum (Ga) (Shen and Buick, 2004), the anaerobic anoxygenic phototrophs most likely converted the abundant compounds sulfide/sulfur by the reverse-operating enzymes of the sulfate reduction pathway. During the Neoproterozoic, the atmospheric oxygen increased to $> 10\%$ of the present levels until 1.05 Ga that resulted in (i) the deepening of the oxic/anoxic interface in the ocean, (ii) the oxygenation of coastal marine sediments, and (iii) decreased levels of sulfide while less reduced inorganic sulfur compounds like thiosulfate became more abundant (Canfield and Teske, 1996; Canfield, 2005). This change in the oxidation state of Earth promoted the evolution and diversification of non-photosynthetic, facultative aerobic or even strict aerobic SOB with a wide-scale initiation of the oxidative sulfur cycle postulated to have occurred lately in the Proterozoic at 0.75–0.62 Ga (Canfield and Teske, 1996). Novel pathways that allowed the usage of the less reduced inorganic sulfur compounds as respiratory electron donor evolved simultaneously in the non-photosynthetic SOB. With regard to the SoxB phylogeny, the Sox enzyme system might have originated in an aerobic, chemotrophic proteobacterial SOB that lacked the reverse sulfate reduction pathway and became widespread among the thiosulfate-utilizing *Proteobacteria*. The reverse sulfate reduction pathway persisted in some facultative anaerobic, chemolithoautotrophic SOB groups (e.g. in *Thiobacillus*, *Thiothrix*, invertebrate symbionts and their free-living relatives) that employed the branched oxidation pathway for thiosulfate oxidation. In adaptation to the changing environmental conditions, the members of the anaerobic anoxygenic phototrophic SOB lineages acquired novel pathways that allowed thiosulfate utilization, e.g. the *sox* gene cluster by lateral transfer from chemotrophic SOB.

Experimental procedures

Microorganisms

The investigated reference strains of photo- and chemotrophic SOB (listed in Table 1) were obtained from the DSMZ (Braunschweig, Germany) as actively growing cultures. Genomic DNA of green sulfur bacteria and several purple sulfur bacteria were received from the culture collection of J. Imhoff, University of Kiel. Extracted genomic DNA of tissue material was provided by N. Dubilier (*Inanidrilus* spp., *B. azoricus*, *B. brevior*), A. D. Nussbauer (*R. pachyptila*, *B. thermophilus*, *C. magnifica*, *Oasisia* sp.) and C. Borowski (*I. nautilei*). Harvested cells of *Beggiatoa* spp., *Aquaspirillum* spp., *Macromonas bipunctata* strain D-408 and *Spirochaeta* spp. were received from G. Dubinina. The SOB strain 'manganese crust' was isolated from enrichment cultures of sediment and seawater samples of the Caribbean Sea (Caribflux project, SO-154).

DNA isolation

Genomic DNA from the investigated reference strains was obtained by applying the DNAeasy Kit (Qiagen, Hilden, Germany) or the NUCLEOBOND® Kit (MACHEREY-NAGEL, Düren, Germany) according to the manufacturer's instructions. The DNA concentration and quality was estimated spectrophotometrically, while its integrity was examined visually by gel electrophoresis on 0.8% (w/v) agarose gels run in 1× Tris-borate-EDTA (TBE) buffer and followed by ethidium bromide staining ($0.5\ \mu g\ ml^{-1}$).

Polymerase chain reaction (PCR) amplification of soxB *and 16S rRNA genes*

Amplification of the *soxB* gene fragments was performed using the primer sets (Table 2) and PCR protocols according to Petri *et al*. (2001). Reaction mixtures (total volume of $50\ \mu l$) contained $5\ \mu l$ 10× RED*Taq* PCR reaction buffer, $5\ \mu l$ 10× BSA solution ($3\ mg\ ml^{-1}$), $200\ \mu M$ (dNTPs) mixture, $1\ \mu M$ of each primer, 2.5 U RED*Taq* DNA polymerase and 10–100 ng genomic DNA from the reference strains as template. 16S rRNA gene fragments were amplified using the primer sets GM3F/GM4R and GM5F-GC clamp/907R [for subsequent denaturing gradient gel electrophoresis (DGGE) analysis] with the PCR conditions as described elsewhere (Muyzer *et al*., 1995).

Cloning of PCR products

Cloning assays of 16S rDNA amplicons and subsequent ARDRA analyses of the recombinant plasmids were performed as described elsewhere (Meyer and Kuever, 2007a).

Double gradient (DG)-DGGE analysis of PCR-amplified 16S rRNA gene fragments

For DG-DGGE analysis, an acrylamide gradient from 6% to 8% acrylamide/bis-acrylamide stock solution, 37.5:1 (v/v) (Bio-Rad), was superimposed over a co-linear denaturant gradient from 20% to 70% of denaturant [100% denaturant corresponds to 7 M urea and 40% formamide (v/v), deionized with AG501-X8 mixed bed resin (Bio-Rad)]. Gradients were formed using a Bio-Rad Gradient Former Model 385. polymerase chain reaction (PCR) samples were applied to the gels in aliquots of 20 µl per lane. Further analysis was performed using the D-CODE™ and D-GENE™ systems (Bio-Rad) for electrophoresis runs in 1× Tris-acetate-EDTA (TAE) buffer at 60°C for 3.5 h at 200 V as previously described by Muyzer *et al*. (1995). After staining with ethidium bromide (0.5 µg ml^{-1}), DNA bands were visualized on a UV transillumination table (Biometra, Göttingen, Germany), excised from the polyacrylamide gel, eluted in 50 µl Tris-HCl, pH 8.0, and re-amplified using the original PCR conditions and primer pair without GC-clamp.

Nucleotide sequencing

The *soxB* and 16S rDNA amplicons of expected size were purified using either the QIAquick PCR purification, the QIAquick gel extraction kit (Qiagen, Hilden, Germany) or the Perfectprep gel cleanup sample kit (Eppendorf, Hamburg, Germany) following the supplier's recommendations. The PCR products were directly sequenced in both directions using the respective amplification primers and the ABI BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, USA). Sequencing reactions were run on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems).

Sequence analysis tools and phylogeny inference

The DNA sequence data of the *soxB* amplicons from each SOB reference strain were assembled with subsequent manual correction using the sequence alignment editor program Bioedit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). BLAST searches for homologous sequences of SoxB in the public databases were performed at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). Searches on the preliminary sequence data of accessible SOB genomes were performed at The Institute for Genomic Research website (http://www.tigr.org) and at the DOE Joint Genome Institute website (http://img.jgi.doe.gov/cgi-bin/pub/main.cgi). The SoxB partial sequences obtained in this study and the complete sequences of the public databases were automatically aligned using the web server Tcoffee@igs (http://igs-server.cnrs-mrs.fr/Tcoffee/). The corresponding nucleic acid sequences of the *soxB* gene fragments were aligned based on the manually corrected amino acids alignment.

The phylogenetic analyses were based on a dataset of (i) 67 full-length SoxB sequences from publicly available genome data of SOB (Table 4), (ii) 7 partial sequences of chemotrophic SOB retrieved from the study of Petri and coworkers (2001), and (iii) 50 novel partial sequences obtained in this study (Table 1). Alignment regions of ambiguous homology as well as indels not present in all investigated sequences were omitted. Unrooted phylogenetic trees were constructed using the tree inference methods included in the ARB software package (http://www.arb-home.de) (distance matrix, neighbour-joining, Fitch; maximum parsimony, ProPars; maximum likelihood, ProML) on the basis of 118 SoxB sequences with 203 compared amino acid positions respectively. The trees were calculated using the global rearrangement, randomized species input order options and JTT matrix as amino-acid replacement model. The robustness of phylogenetic trees was tested by bootstrap analysis with 100 re-samplings. Short partial sequences were individually added to the initial trees using the QUICK_ADD parsimony tool of ARB without allowing changes in the overall tree topology. Finally, a SoxB-based consensus tree was constructed after comparing the topologies of the phylogenetic trees calculated by distance matrix, maximum parsimony and maximum likelihood analyses. The 16S rRNA gene-based consensus tree was generated as described for the SoxB phylogeny inference (16S rRNA gene sequences were obtained from the public databases).

Southern blot analysis

Identical amounts of genomic DNA (5 µg) from sulfur-oxidizing and sulfate-reducing bacteria (Table 3) were digested at 37°C with HindIII and EcoRI overnight, precipitated by ethanol, electrophoresed on 0.8% 1× TAE buffer at 100 V for 3 h, transferred to positively charged nylon membranes (Hybond N + filter, Amersham) by capillary neutral transfer and immobilized by UV cross-linking (Transilluminator, Biometra). The DNA probes for *soxB* genes (0.7 kb in length) were radioactively labelled with [α-^{32}P]dCTP by the random priming method using the HexaLabel™ DNA Labeling Kit (MBI Fermentas) according to the manufacturer's directions. The membranes were placed into glass hybridization bottles and prehybridized in 5× SSC (1× SSC is 0.15 M NaCl, 0.015 M Na-citrate, pH 8.0), 50% formamide, 0.1% sarcosyl, 7% SDS, 50 mM phosphate buffer, pH 7.0 and 2% casein ('Church' hybridization solution) at 50°C for 1 h in a hybridization oven (Biometra). Subsequently, a freshly denaturated, labelled DNA probe was added to the prehybridization solution followed by incubation for 12–16 h at 50°C under slow-speed rotation. The membranes were washed twice at 50°C for 30 min in 0.1× SSC-0.1% SDS, exposed to PhosphorImaging screen cassettes (Molecular Dynamics, Krefeld, Germany), scanned with a Typhoon Variable Mode Imager and processed with Image Quant software (Amersham). The membranes were stripped by two incubations for 15 min in probe-stripping solution (consisting of 0.4 M NaOH and 0.1% SDS) at 37°C under permanent agitation and re-probed, starting from the prehybridization step of the hybridization procedure.

GenBank accession numbers

The nucleotide sequence data reported in this study have been submitted to GenBank and are available under accession number EF618568-EF618617.

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References

Appia-Ayme, C., Little, P.J., Matsumoto, Y., Leech, A.P., and Berks, B.C. (2001) Cytochrome complex essential for photosynthetic oxidation of both thiosulfate and sulfide in *Rhodovulum sulfidophilum*. *J Bacteriol* **183:** 6107–6118.

Beller, H.R., Letain, T.E., Chakicherla, A., Kane, S.R., Legler, T.C., and Coleman, M.A. (2006) Whole-genome transcriptional analysis of chemolithoautotrophic thiosulfate oxidation by *Thiobacillus denitrificans* under aerobic versus denitrifying conditions. *J Bacteriol* **188:** 7005–7015.

Boucher, Y., Douady, C.J., Papke, R.T., Walsh, D.A., Boudreau, M.E.R., Nesbo, C.L., *et al.* (2003) Lateral gene transfer and the origins of prokaryotic groups. *Annu Rev Genet* **37:** 283–328.

Brinkhoff, T., Muyzer, G., Wirsen, C.O., and Kuever, J. (1999) *Thiomicrospira kuenenii* sp. nov. & *Thiomicrospira frisia* sp. nov., two mesophilic obligately chemolithoautotrophic sulfur-oxidizing bacteria isolated from an intertidal mud flat. *Int J Syst Bacteriol* **49:** 385–392.

Brocks, J.J., Love, G.D., Summons, R.E., Knoll, A.H., Logan, G.A., and Bowden, S.A. (2005) Biomarker evidence for green and purple sulphur bacteria in a stratified Palaeoproterozoic sea. *Nature* **437:** 866–870.

Brune, D.C. (1995) Sulfur compounds as photosynthetic electron donors. In *Anoxygenic Photosynthetic Bacteria*. Blankenship, R.E., Madigan, M.T., and Bauer, C.E. (eds). Dordrecht, The Netherlands: Kluwer Academic Publishers, pp. 847–870.

Brüser, T., Lens, P.N.L., and Trüper, H.G. (2000) The biological sulfur cycle. In *Environmental Technologies to Treat Sulfur Pollution*. Lens, P.N.L., and Pol, L.H. (eds). London, UK: IWA Publishing, pp. 47–86.

Buchan, A., Gonzalez, J.M., and Moran, M.A. (2005) Overview of the marine Roseobacter lineage. *Appl Environ Microbiol* **71:** 5665–5677.

Campbell, B.J., Engel, A.S., Porter, M.L., and Takai, K. (2006) The versatile epsilon-proteobacteria: key players in sulphidic habitats. *Nat Rev Genet* **4:** 458–468.

Canfield, D.E. (2005) The early history of atmospheric oxygen: Homage to Robert A. Garrels. *Annu Rev Earth Planet Sci* **33:** 1–36.

Canfield, D.E., and Teske, A. (1996) Late Proterozoic rise in atmospheric oxygen concentration inferred from phylogenetic and sulphur-isotope studies. *Nature* **382:** 127–132.

Cavanaugh, C.M., McKiness, Z.P., Newton, I.L.G., and Stewart, F.J. (2004) Marine chemosynthetic symbioses. In *The Prokaryotes: An Evolving Electronic Resource for the Microbial Community*. Dworkin, M., Falkow, E., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds). [WWW document]. URL http://link.springer-ny.com/link/service/books/10125/index.htm.

Dahl, C., Rakhely, G., Pott-Sperling, A.S., Fodor, B., Takacs, M., Toth, A., *et al.* (1999) Genes involved in hydrogen and sulfur metabolism in phototrophic sulfur bacteria. *FEMS Microbiol Lett* **180:** 317–324.

Dahl, C., Engels, S., Pott-Sperling, A.S., Schulte, A., Sander, J., Lubbe, Y., *et al.* (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.

Dubinina, G.A., Grabovich, M.Y., and Chernyshova, Y.Y. (2004) The role of oxygen in the regulation of the metabolism of aerotolerant Spirochetes, a major component of '*Thiodendron*' bacterial sulfur mats. *Microbiology* **73:** 725–733.

Dutilh, B.E., Huynen, M.A., Bruno, W.J., and Snel, B. (2004) The consistent phylogenetic signal in genome trees revealed by reducing the impact of noise. *J Mol Evol* **58:** 527–539.

Epel, B., Schafer, K.O., Quentmeier, A., Friedrich, C., and Lubitz, W. (2005) Multifrequency EPR analysis of the dimanganese cluster of the putative sulfate thiohydrolase SoxB of *Paracoccus pantotrophus*. *J Biol Inorg Chem* **10:** 636–642.

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

Friedrich, C.G., Bardischewsky, F., Rother, D., Quentmeier, A., and Fischer, J. (2005) Prokaryotic sulfur oxidation. *Curr Opin Microbiol* **8:** 253–259.

Hanson, T.E., and Tabita, F.R. (2003) Insights into the stress response and sulfur metabolism revealed by proteome analysis of a *Chlorobium tepidum* mutant lacking the Rubisco-like protein. *Photosynthesis Res* **78:** 231–248.

Hensen, D., Sperling, D., Trüper, H.G., Brune, D.C., and Dahl, C. (2006) Thiosulphate oxidation in the phototrophic sulphur bacterium *Allochromatium vinosum*. *Mol Microbiol* **62:** 794–810.

Howarth, R., Unz, R.F., Seviour, E.M., Seviour, R.J., Blackall, L.L., Pickup, R.W., *et al.* (1999) Phylogenetic relationships of filamentous sulfur bacteria (*Thiothrix* spp. & Eikelboom type 021N bacteria) isolated from wastewater-treatment plants and description of *Thiothrix eikelboomii* sp. nov., *Thiothrix unzii* sp. nov., *Thiothrix fructosivorans* sp. nov. and *Thiothrix defluvii* sp. nov. *Int J Syst Bacteriol* **49:** 1817–1827.

Huber, H., and Stetter, K.O. (1989) *Thiobacillus prosperus* sp. nov., represents a new group of halotolerant metal-mobilizing bacteria isolated from a marine geothermal field. *Arch Microbiol* **151:** 479–485.

Imhoff, J.F. (1999) The family *Ectothiorhodospiraceae*. In *The Prokaryotes: An Evolving Electronic Resource for the Microbial Community*. Dworkin, M., Falkow, E., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds). [WWW document]. URL http://link.springer-ny.com/link/service/books/10125/index.htm.

Imhoff, J.F. (2001a) The *Chromatiaceae*. In *The Prokaryotes: An Evolving Electronic Resource for the Microbial Community*. Dworkin, M., Falkow, E., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds). [WWW document]. URL http://link.springer-ny.com/link/service/books/10125/index.htm.

Imhoff, J.F. (2001b) The phototrophic alpha-Proteobacteria. In *The Prokaryotes: An Evolving Electronic Resource for the Microbial Community*. Dworkin, M., Falkow, E., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds). [WWW document]. URL http://link.springer-ny.com/link/service/books/10125/index.htm.

Imhoff, J.F. (2001c) The phototrophic beta-Proteobacteria. In *The Prokaryotes: An Evolving Electronic Resource for the Microbial Community*. Dworkin, M., Falkow, E., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds). [WWW document]. URL http://link.springer-ny.com/link/service/books/10125/index.htm.

Imhoff, J.F. (2003) Phylogenetic taxonomy of the family *Chlorobiaceae* on the basis of 16S rRNA and *fmo* (Fenna Matthews-Olson protein) gene sequences. *Int J Syst Evol Microbiol* **53:** 941–951.

Ito, T., Sugita, K., and Okabe, S. (2004) Isolation, characterization, and in situ detection of a novel chemolithoautotrophic sulfur-oxidizing bacterium in wastewater biofilms growing under microaerophilic conditions. *Appl Environ Microbiol* **70:** 3122–3129.

Joergensen, B.B., and Nelson, D.C. (2004) Sulfide oxidation in marine sediments: geochemistry meets microbiology. In *Sulfur Biogeochemistry – Past and Present*. Amend, J.P., Edwards, K.J., and Lyons, T.W. (eds). Boulder, Colorado, USA: Geological Society of America, pp. 63–81.

Kappler, U., and Dahl, C. (2001) Enzymology and molecular biology of prokaryotic sulfite oxidation. *FEMS Microbiol Lett* **203:** 1–9.

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

Kelly, D.P., and Wood, A.P. (2000) Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov. and *Thermithiobacillus* gen. nov. *Int J Syst Evol Microbiol* **50:** 511–516.

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

Kletzin, A., Urich, T., Muller, F., Bandeiras, T.M., and Gomes, C.M. (2004) Dissimilatory oxidation and reduction of elemental sulfur in thermophilic archaea. *J Bioenerg Biomembr* **36:** 77–91.

Kuever, J., Sievert, S.M., Stevens, H., Brinkhoff, T., and Muyzer, G. (2002) Microorganisms of the oxidative and reductive part of the sulfur cycle at a shallow-water hydrothermal vent in the Aegean Sea (Milos, Greece). *Cah Biol Mar* **43:** 413–416.

Kwok, S., Kellogg, D.E., McKinney, N., Spasic, D., Goda, L., Levenson, C., and Sninsky, J.J. (1990) Effects of primer-template mismatches on the polymerase chain reaction. *Nucleic Acids Res* **18:** 999–1005.

Mendez-Alvarez, S., Pavon, V., Esteve, I., Guerrero, R., and Gaju, N. (1994) Transformation of *Chlorobium limicola* by a plasmid that confers the ability to utilize thiosulfate. *J Bacteriol* **176:** 7395–7397.

Meulenberg, R., Scheer, E.J., Pronk, J.T., Hazeu, W., Bos, P., and Kuenen, J.G. (1993) Metabolism of tetrathionate in *Thiobacillus acidophilus*. *FEMS Microbiol Lett* **112:** 167–172.

Meyer, B., and Kuever, J. (2007a) Phylogeny of the alpha and beta subunits of the dissimilatory adenosine-5′-phosphosulfate (APS) reductase from sulfate-reducing prokaryotes – origin and evolution of the dissimilatory sulfate-reduction pathway. *Microbiology* **153:** 2026–2044.

Meyer, B., and Kuever, J. (2007b) Molecular analysis of the distribution and phylogeny of dissimilatory adenosine-5′-phosphosulfate reductase encoding genes (*aprBA*) among sulfur-oxidizing prokaryotes. *Microbiology*: in press.

Mukhopadhyaya, P.N., Deb, C., Lahiri, C., and Roy, P. (2000) A *soxA* gene, encoding a diheme cytochrome c, and a sox locus, essential for sulfur oxidation in a new sulfur lithotrophic bacterium. *J Bacteriol* **182:** 4278–4287.

Muyzer, G., Teske, A., Wirsen, C.O., and Jannasch, H.W. (1995) Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Arch Microbiol* **164:** 165–172.

Nelson, D.C., and Fisher, C.R. (1995) Chemoautotrophic and methanoautotrophic endosymbiontic bacteria at deep-sea vents and seeps. In *Deep Sea Hydrothermal Vents*. Karl, D.M. (ed.). Boca Raton, Florida, USA: CRC Press, pp. 125–167.

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

Pott, A.S., and Dahl, C. (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. *Microbiol* **144:** 1881–1894.

Pronk, J.T., Meulenberg, R., Hazeu, W., Bos, P., and Kuenen, J.G. (1990) Oxidation of reduced anorganic sulphur compounds by acidophilic thiobacilli. *FEMS Microbiol Rev* **75:** 293–306.

Qureshi, S., Richards, B.K., Hay, A.G., Tsai, C.C., McBride, M.B., Baveye, P., and Steenhuis, T.S. (2003) Effect of microbial activity on trace element release from sewage sludge. *Environ Sci Technol* **37:** 3361–3366.

Reysenbach, A.L., Banta, A.B., Boone, D.R., Cary, S.C., and Luther, G.W. (2000) Biogeochemistry – microbial essentials at hydrothermal vents. *Nature* **404:** 835–835.

Robertson, L.A., and Kuenen, G.J. (2002) The genus *Thiobacillus*. In *The Prokaryotes: An Evolving Electronic Resource for the Microbial Community*. Dworkin, M., Falkow, E., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds). [WWW document]. URL http://link.springer-ny.com/link/service/books/10125/index.htm.

Rohwerder, T., and Sand, W. (2003) The sulfane sulfur of persulfides is the actual substrate of the sulfur-oxidizing enzymes from *Acidithiobacillus* and *Acidiphilium* spp. *Microbiology* **149:** 1688–1709.

Sanchez, O., Ferrera, I., Dahl, C., and Mas, J. (2001) In vivo role of adenosine-5′-phosphosulfate reductase in the purple sulfur bacterium *Allochromatium vinosum*. *Arch Microbiol* **176:** 301–305.

Schippers, A. (2004) Biogeochemistry of metal sulfide oxidation in mining environments, sediments and soils. In *Sulfur Biogeochemistry – Past and Present*. Amend, J.P., Edwards, K.J., and Lyons, T.W. (eds). Boulder, Colorado, USA: Geological Society of America, pp. 49–62.

Shen, Y., Knoll, A.H., and Walter, M.R. (2003) Evidence for low sulphate and anoxia in a mid-Proterozoic marine basin. *Nature* **423:** 632–635.

Shen, Y.N., and Buick, R. (2004) The antiquity of microbial sulfate reduction. *Earth Sci Rev* **64:** 243–272.

Simsek, M., and Adnan, H. (2000) Effect of single mismatches at 3′-end of primers on polymerase chain reaction. *Med Sci* **2:** 11–14.

Sorokin, D.Y. (2003) Oxidation of inorganic sulfur compounds by obligately organotrophic bacteria. *Microbiology* **72:** 641–653.

Takai, K., Campbell, B.J., Cary, S.C., Suzuki, M., Oida, H., Nunoura, T., *et al.* (2005) Enzymatic and genetic characterization of carbon and energy metabolisms by deep-sea hydrothermal chemolithoautotrophic isolates of *Epsilonproteobacteria*. *Appl Environ Microbiol* **71:** 7310–7320.

Takai, K., Suzuki, M., Nakagawa, S., Miyazaki, M., Suzuki, Y., Inagaki, F., and Horikoshi, K. (2006) *Sulfurimonas paravinellae* sp. nov., a novel mesophilic, hydrogen- and sulfur-oxidizing chemolithoautotroph within the *Epsilonproteobacteria* isolated from a deep-sea hydrothermal vent polychaete nest, reclassification of *Thiomicrospira denitrificans* as *Sulfurimonas denitrificans* comb. nov. and emended description of the genus *Sulfurimonas*. *Int J Syst Evol Microbiol* **56:** 1725–1733.

Teske, A., and Nelson, D.C. (2004) The genera *Beggiatoa* and *Thioploca*. In *The Prokaryotes: An Evolving Electronic Resource for the Microbial Community*. Dworkin, M., Falkow, E., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds). [WWW document]. URL http://link.springer-ny.com/link/service/books/10125/index.htm.

Zopfi, J., Ferdelman, T.G., and Fossing, H. (2004) Distribution and fate of sulfur intermediates– sulfite, tetrathionate, thiosulfate, and elemental sulfur – in marine sediments. In *Sulfur Biogeochemistry – Past and Present*. Amend, J.P., Edwards, K.J., and Lyons, T.W. (eds). Boulder, Colorado, USA: Geological Society of America, pp. 97–116.

Supplementary material

The following supplementary material is available for this article online:

Table S1. SoxB alignment showing indels among selected representatives of the major phylogenetic SOB lineages, supporting the inferred relationships including the postulated LGTs of *soxB* among the investigated SOB species. Amino acid positions according to the enumeration of *Paracoccus denitrificans* str. GB17 proteins. Identical indel positions in SoxB sequences are indicated by boxes.

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