Microbiology (2007), **153**, 2026–2044 DOI 10.1099/mic.0.2006/003152-0

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

Birte Meyer and Jan Kuever†

Max-Planck-Institute for Marine Microbiology, Celsiusstrasse 1, D-28359 Bremen, Germany

Correspondence
Jan Kuever
kuever@mpa-bremen.de

Newly developed PCR assays were used to PCR-amplify and sequence fragments of the dissimilatory adenosine-5′-phosphosulfate (APS) reductase genes (*aprBA*) comprising nearly the entire gene locus (2·2–2·4 kb, equal to 92–94 % of the protein coding sequence) from 75 sulfate-reducing prokaryotes (SRP) of a taxonomically wide range. Comparative phylogenetic analysis included all determined and publicly available AprBA sequences from SRP and selected homologous sequences of sulfur-oxidizing bacteria (SOB). The almost identical AprB and AprA tree topologies indicated a shared evolutionary path for the *aprBA* among the investigated SRP by vertical inheritance and concomitant lateral gene transfer (LGT). The topological comparison of AprB/A- and 16S rRNA gene-based phylogenetic trees revealed novel LGT events across the SRP divisions. Compositional gene analysis confirmed *Thermacetogenium phaeum* to be the first validated strain affected by a recent lateral transfer of *aprBA* as a putative effect of long-term co-cultivation with a *Thermodesulfovibrio* species. Interestingly, the Apr proteins of SRP and SOB diverged into two phylogenetic lineages, with the SRP affiliated with the green sulfur bacteria, e.g. *Chlorobaculum tepidum*, while the *Allochromatium vinosum*-related sequences formed a distinct group. Analysis of genome data indicated that this phylogenetic separation is also reflected in the differing presence of the putative proteins functionally associated with Apr, QmoABC complex (quinone-interacting membrane-bound oxidoreductase) and AprM (transmembrane protein). Scenarios for the origin and evolution of the dissimilatory APS reductase are discussed within the context of the dissimilatory sulfite reductase (DsrAB) phylogeny, the appearance of QmoABC and AprM in the SRP and SOB genomes, and the geochemical setting of Archean Earth.

Received 5 October 2006
Revised 22 February 2007
Accepted 21 March 2007

INTRODUCTION

Microbial sulfate respiration is an ancient metabolic pathway of energy conservation, which probably originated as early as 3·47 billion years ago (Shen *et al.*, 2001). Despite its suggested antiquity, the capability for dissimilatory sulfate reduction is patchily distributed, and occurs solely within members of six bacterial and two distinct archaeal lineages (Itoh *et al.*, 1999; Mori *et al.*, 2003; Moussard *et al.*, 2004; Rabus *et al.*, 1999). In all recognized sulfate-reducing prokaryotes (SRP), the dissimilatory process is mediated by three key enzymes. After the activation of the chemically inert sulfate to adenosine-5′-phosphosulfate (APS) by ATP sulfurylase (Sat), the second enzyme, APS reductase (Apr), converts APS to AMP and sulfite, which is finally reduced to sulfide by the activity of the sulfite reductase (Dsr) (Rabus *et al.*, 1999). Homologous proteins are also present in the anoxygenic photolithotrophic and chemolithotrophic sulfur-oxidizing bacteria (SOB) (Dahl & Trüper, 1994; Hipp *et al.*, 1997; Schedel & Trüper, 1979, 1980; Sperling *et al.*, 1998; Trüper & Fischer, 1982). Indeed, earlier studies have confirmed that the dissimilatory APS

†Present address: Bremen Institute for Materials Testing, Paul-Feller-Strasse 1, D-28199 Bremen, Germany.

Abbreviations: APS, adenosine-5′-phosphosulfate; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; LGT, lateral gene transfer; SOB, sulfur-oxidizing bacteria; SRB, sulfate-reducing bacterium/a; SRP, sulfate-reducing prokaryotes; TIGR, The Institute for Genomic Research.

The GenBank/EMBL/DDBJ accession numbers for the nucleotide sequence data reported in this study are EF442876–EF442976 (*apr*) and EF442977–EF442994 (16S rRNA).

Supplementary data are available with the online version of this paper.

reductases are highly conserved among SRP and SOB and form heterodimers with one alpha subunit (75–80 kDa, one FAD) and one beta subunit (18–23 kDa, two [4Fe–4S] centres) which are encoded by the *aprBA* gene loci (Fritz *et al.*, 2000; Hipp *et al.*, 1997; Lampreia *et al.*, 1994; Molitor *et al.*, 1998; Speich *et al.*, 1994). In contrast, the proteins that mediate the electron transport between the cytoplasmic AprBA and DsrAB and the quinol/quinone pool in the membrane are still poorly characterized. However, experimental evidence is accumulating that the membrane-bound redox complexes HmeABCDE [heterodisulfide reductase (Hdr)-like menaquinol-oxidizing enzyme] and homologous DsrMKJOP, as well as QmoABC (quinone-interacting membrane-bound oxidoreductase), are involved (Dahl *et al.*, 2005; Mander *et al.*, 2002; Pires *et al.*, 2003, 2006; Sander *et al.*, 2006). The Qmo redox complex from *Desulfovibrio desulfuricans* ATCC 27774 has been described as consisting of two soluble, HdrA-homologous, FAD-containing proteins, QmoA and QmoB, and membrane integral, HdrC/E-homologous protein, QmoC, containing two haem *b* groups and a putative quinone-binding site. Based on experimental results with *Desulfovibrio desulfuricans* ATCC 27774, the QmoABC complex has been postulated to be the missing link between the membrane menaquinol/menaquinone pool and the cytoplasmic reduction of APS by acting as a menaquinol/APS reductase oxidoreductase (Pires *et al.*, 2003).

Although lateral gene transfer (LGT) between distantly related phylogenetic lineages and domains is well-documented, especially for genes that encode proteins of metabolic pathways (Boucher *et al.*, 2003), the general impact of LGT as a major driving force in the genome evolution of prokaryotes is still debated (Daubin & Ochman, 2004; Gogarten & Townsend, 2005; Jain *et al.*, 2003; Kurland *et al.*, 2003; Lerat *et al.*, 2005). The evolution of the sulfate-respiration process in SRP has primarily been investigated by comparative phylogenetic studies of the dissimilatory sulfite reductase DsrAB, and this has confirmed the occurrence of multiple events of LGT of *dsrAB* among members of this physiological group (Klein *et al.*, 2001; Mussmann *et al.*, 2005; Zverlov *et al.*, 2005). A widespread dispersal via 'metabolic islands' has recently been discussed as responsible for the polyphyletic distribution of this metabolic trait (Klein *et al.*, 2001; Mussmann *et al.*, 2005). In contrast to the comprehensive analysis of DsrAB phylogeny, knowledge concerning the evolution of the dissimilatory APS reductase is currently restricted to a single phylogenetic study based on the limited sequence information of a minor part of the *aprA* gene-coding region (~0·9 kb) from 60 taxonomically different SRP species (Friedrich, 2002). The AprA tree topology of that study differed partially from the 16S rRNA gene-based tree, and led the author to suggest frequent events of inter- and intradomain LGT of the *apr* genes involving members of the Gram-positive sulfate-reducing bacteria (SRB), *Syntrophobacteraceae*, *Syntrophaceae* and *Archaeoglobus*, as well as the thermophilic *Thermodesulfovibrio islandicus* and representatives of the genus *Thermodesulfobacterium* (Friedrich, 2002).

The aim of this study was to increase the available genetic information for the *apr* gene locus by developing new PCR primer pairs for amplification and sequencing of nearly the entire coding region of the dissimilatory APS reductase (*aprBA*) genes from reference strains of all currently known SRP lineages. The phylogeny of both subunits AprB and AprA of the dissimilatory APS reductase from 103 different SRP (including some selected sequences of reverse-operating APS reductases of SOB) was compared with their 16S rRNA gene-based phylogeny to reveal novel events of LGT among the examined sulfate-reducing species. In addition, the results of the Apr phylogenetic analyses are discussed within the context of (1) the DsrAB phylogeny, (2) the collected genomic data concerning the presence and genomic arrangement of genes coding for putative functionally associated proteins (Qmo complex, AprM) at the *apr* gene locus of SRP and SOB, and (3) the geochemical data, in order to elucidate the origin and evolution of dissimilatory sulfate reduction/sulfite oxidation in prokaryotes.

METHODS

Micro-organisms. The investigated SRP reference strains (listed in Table 1) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) either as lyophilized or as actively growing cultures. If necessary, strains were cultivated as recommended by the DSMZ. Actively growing cultures of the following reference strains were provided by O. Kniemeyer (SRB strain EbS7), A. Galushko (strain NaphS2), G. Harms (strain mXyS1), H. T. Dinh ('*Desulfovibrio ferrophilus*' strain IS5, '*Desulfobacterium corrodens*' strain IS4 and SRB strain QLNR1), K. Nauhaus (*Desulfofrigus* sp. strain HRS-LA3X and *Desulfovibrio* sp. strain HRS-LA4), M. Könnecke (SRB strain JHA1) and J. Detmers (*Desulfovibio* sp. strain JD-160, *Desulfotomaculum* sp. strains JD-175 and JD-176) at the Max-Planck-Institute for Marine Microbiology, Bremen, and by H. P. Goorissen (*Desulfotomaculum solfataricum*) and T. A. Hansen (*Desulfobacterium niacini* strain PM4) at the University of Groningen. *Desulfovibrio* sp. strains LB1/LA1(H_2)/LA2/49MC/sponge tissue 85CD as well as *Desulfobulbus* sp. strain LB2 were isolated from sediment and seawater samples of the Caribbean Sea (RV Sonne cruise SO-154, Caribflux project).

DNA isolation. All general molecular techniques were performed according to the described standard methods (Sambrook *et al.*, 1989). Genomic DNAs were extracted from the investigated strains using the DNAeasy kit (Qiagen) according to the manufacturer's instructions. The DNA concentration was measured using a spectrophotometer. DNA quality was checked by PCR amplification with the 16S rRNA gene-targeting primer sets GM3F/GM4R and Arch21F/Arch958R, as described elsewhere (DeLong, 1992; Muyzer *et al.*, 1995).

PCR primers. Two sets of degenerate primers that anneal to conserved *aprBA* gene regions of SRP (Table 2) were newly designed based on comparison of *Desulfovibrio vulgaris*, *Desulfovibrio desulfuricans*, *Archaeoglobus fulgidus* (see Table 1 for GenBank accession numbers), *Allochromatium vinosum* (U84759) and *Chlorobaculum tepidum* (NC_002932) full-length *apr* sequences. Since the *aprA* alignment revealed a limited number of suitable primer target sites and a generally low degree of conserved nucleotide positions in the 3′

Table 1. PCR amplification results of *aprBA* and *aprA* gene fragments from genomic DNA of sulfate-reducing reference strains

Species*	Strain†	PCR product obtained with primer set‡			GenBank accession no.§	
		AprB-1-FW/ AprA-5-RV	AprA-1-FW/ AprA-10-RV	AprA-1-FW/ AprA-11-RV	*aprBA*	16S rRNA
Archaea						
Phylum *Crenarchaeota, Thermoprotei*						
Thermoproteaceae						
Caldivirga maquilingensis	13496^T	− (52)	− (56)	− (50)	−	AB013926
Phylum *Euryarchaeota, Archaeoglobi*						
Archaeaglobaceae						
Archaeoglobus fulgidus	4304^T	+‖		+‖	NC_000917	AE000965
Archaeoglobus profundus	5631^T	+ (60)	ND	+ (62)	**EF442876**	AF297529
Archaeoglobus veneficus	11195^T	+ (60)	ND	+ (62)	**EF442877**	AF418181
Bacteria						
Phylum *Thermodesulfobacteria, Thermodesulfobacteria*						
Thermodesulfobacteriaceae						
Thermodesulfobacterium commune	2178^T	+ (60)	+ (56)	− (50)	**EF442878**	AF418169
Thermodesulfobacterium hveragardense	12571^T	+ (58)	+ (56)	− (50)	**EF442879**	X96725
Thermodesulfatator indicus	15286^T	+ (58)	+ (56)	ND	**EF442880**	AF393376
Phylum *Nitrospira, Nitrospira*						
Nitrospiraceae						
Thermodesulfovibrio yellowstonii	11374^T	+ (60)	+ (62)	− (50)	**EF442881**	L14619
Phylum *Proteobacteria, Deltaproteobacteria*						
Desulfovibrionaceae						
Desulfovibrio acrylicus	10141^T	+ (60)	± (62)	ND	**EF442882**	U32578
Desulfovibrio oxyclinae	11498^T	+ (54)	+ (62)	ND	**EF442883**	U33316
Desulfovibrio desulfuricans strain G20	−	+‖	+‖		NC_007519	CP000112
Desulfovibrio desulfuricans ssp. *desulfuricans* strain Essex	642^T	+‖	+‖		AF226708	AF192153
'*Desulfovibrio ferrophilus*' strain IS5	15579	± (54)	+ (54)	ND	**EF442884**	AY274449
Desulfovibrio piger	749^T	± (56)	+ (62)	ND	**EF442885**	AF192152
Desulfovibrio profundus	11384^T	± (58)	+ (62)	ND	**EF442886**	AF418172
Desulfovibrio salexigens	2638^T	+ (58)	± (62)	ND	**EF442887**	M34401
Desulfovibrio sp.	9953	± (54)	+ (62)	ND	**EF442888**	NA
Desulfovibrio sp. strain HRS-LA4	−	+ (54)	ND	ND	**EF442889**	**EF442986**
Desulfovibrio sp. strain X	−	+ (54)	± (52)	ND	**EF442890**	**EF442979**
Desulfovibrio sp. strain JD-160	−	± (54)	+ (56)	ND	**EF442891**	AF295660
Desulfovibrio sp. strain 49MC	−	+ (54)	ND	ND	**EF442893**	**EF442988**
Desulfovibrio sp. strain LB1	−	+ (54)	ND	ND	**EF442892**	EF614447
Desulfovibrio sp. strain LA1(H_2)	−	+ (54)	ND	ND	**EF442894**	**EF442991**
Desulfovibrio sp. strain LA2	−	± (54)	ND	ND	**EF442895**	**EF442992**
Desulfovibrio sp. strain sponge tissue 85CD	−	± (54)	ND	ND	**EF442896**	**EF442989**
Desulfovibrio sulfodismutans	3969^T	+ (58)	+ (62)	ND	**EF442897**	Y17764
Desulfovibrio vulgaris ssp. *vulgaris* strain Hildenborough	644^T	+‖		+‖	NC_002937	AF418179
Desulfomicrobiaceae						
Desulfocaldus sp. strain Hobo	−	+ (60)	+ (62)	ND	**EF442898**	**EF442977**
Desulfomicrobium baculatum	4028^T	+ (60)	+ (62)	ND	**EF442899**	AF030438
Desulfohalobiaceae						
Desulfohalobium retbaense	5692^T	± (54)	+ (62)	ND	**EF442900**	U48244
Desulfonatronovibrio hydrogenovorans	9292^T	+ (56)	+ (62)	ND	**EF442902**	X99234
Desulfonauticus submarinus	15269^T	± (54)	+ (62)	ND	**EF442903**	AF524933
Desulfothermus naphthae strain TD3	13418^T	+ (58)	+ (62)	ND	**EF442901**	X80922
SRB strain 4206	4206	± (54)	+ (62)	ND	**EF442904**	SS
Desulfonatronumaceae						
Desulfonatronum lacustre	10312^T	ND	+ (62)	ND	**EF442905**	AF418171
Desulfobacteraceae						

Table 1. cont.

Species*	Strain†	PCR product obtained with primer set‡			GenBank accession no.§	
		AprB-1-FW/ AprA-5-RV	AprA-1-FW/ AprA-10-RV	AprA-1-FW/ AprA-11-RV	*aprBA*	16S rRNA
Desulfatibacillum sp. strain Pnd3	–	± (55)	+ (62)	ND	**EF442906**	Y17501
Desulfobacter postgatei	2034^T	± (54)	± (56)	ND	**EF442907**	AF418180
Desulfobacter sp.	2035	+ (55)	± (52)	ND	**EF442908**	M34415
Desulfobacter sp.	2057	+ (55)	± (52)	ND	**EF442909**	M34416
Desulfobacterium autotrophicum	3382^T	+ (58)	± (62)	ND	**EF442910**	AF418177
Desulfobacterium indolicum	3883^T	+ (58)	+ (62)	ND	**EF442911**	AJ237607
Desulfobacterium niacini	2650^T	± (57)	+ (62)	ND	**EF442912**	M34405
Desulfobacterium niacini strain PM4	–	± (54)	+ (62)	ND	**EF442913**	U51845
'Desulfobacterium oleovorans' strain Hxd3	6200^T	± (57)	+ (62)	ND	**EF442914**	Y17698
SRB strain JHA1	–	± (54)	± (56)	ND	**EF442915**	**EF442984**
Desulfobacterium sp.	7120^T	+ (57)	± (56)	ND	**EF442916**	**EF442994**
Desulfobacterium zeppelinii	9120	± (54)	+ (62)	ND	**EF442917**	**EF442983**
Desulfobacula phenolica	3384^T	+ (57)	+ (62)	ND	**EF442918**	AJ237606
Desulfobacula toluolica	7467^T	+ (58)	+ (62)	ND	**EF442919**	AJ441316
Desulfobotulus sapovorans	2055^T	+ (54)	+ (62)	ND	**EF442920**	M34402
Desulfocella sp.	2056^T	± (52)	± (50)	ND	**EF442921**	**EF442981**
Desulfococcus multivorans	2059^T	+ (60)	+ (62)	ND	**EF442922**	AF418173
Desulfococcus sp.	8541	+ (55)	+ (62)	ND	**EF442923**	**EF442980**
Desulfofrigus sp. strain HRS-LA3X	–	+ (54)	+ (56)	ND	**EF442924**	**EF442987**
Desulfonema limicola	2076^T	+ (57)	+ (56)	ND	**EF442925**	U45990
Desulfonema magnum	2077^T	+ (60)	+ (56)	ND	**EF442926**	U45989
Desulforegula conservatrix	13587^T	+ (58)	+ (62)	ND	**EF442927**	AF243334
Desulfosarcina variabilis	2060^T	± (58)	+ (62)	ND	**EF442928**	M34407
Desulfospira joergensenii	10085^T	± (58)	+ (62)	ND	**EF442929**	X99637
Desulfotignum balticum	7044^T	+ (57)	+ (62)	ND	**EF442930**	AF418176
Desulfobulbaceae						
Desulfobacterium catecholicum	3882^T	+ (58)	± (50)	ND	**EF442931**	AJ237602
Desulfobacterium catecholicum strain Kette	–	+ (57)	± (50)	ND	**EF442932**	**EF442982**
'Desulfobacterium corrodens' strain IS4	15630	+ (58)	± (52)	ND	**EF442933**	AY274450
Desulfobulbus marinus	2058^T	± (54)	+ (62)	ND	**EF442934**	M34411
Desulfobulbus propionicus	2032^T	+ (58)	± (62)	ND	**EF442935**	M34410
Desulfobulbus sp.	2033	+ (57)	+ (62)	ND	**EF442936**	**EF442993**
Desulfobulbus sp. strain LB2	–	± (54)	ND	ND	**EF442937**	**EF442990**
Desulfocapsa thiozymogenes	7269^T	± (58)	+ (62)	ND	**EF442938**	X95181
Desulfofustis glycolicus	9705^T	+ (58)	+ (62)	ND	**EF442939**	X99707
Desulforhopalus vacuolatus	9700^T	± (57)	± (62)	ND	**EF442940**	L42613
Desulfotalea psychrophila	12343^T	+ (55)	± (52)	ND	NC_006138	AF099062
SRB strain QLNR1	–	+ (58)	± (52)	ND	**EF442941**	**EF442985**
Uncertain affiliation						
Desulfobacterium anilini	4660^T	± (54)	– (52)	± (62)	**EF442942**	AJ237601
SRB strain EbS7	15769	± (55)	– (54)	+ (62)	**EF442943**	AJ430774
SRB strain NaphS2	14454	± (54)	– (54)	+ (62)	**EF442944**	AJ132804
SRB strain mXyS1	12567	+ (58)	– (52)	+ (62)	**EF442945**	AJ006853
Desulfarculaceae						
Desulfarculus baarsii	2075^T	+ (58)	ND	± (62)	**EF442946**	AF418174
Syntrophaceae						
Desulfobacca acetoxidans	11109^T	± (58)	± (50)	± (50)	**EF442947**	AF002671
Desulfomonile tiedjei	6799^T	± (55)	– (50)	± (56)	**EF442948**	M26635
Syntrophobacteraceae						
Desulforhabdus amnigena	10338^T	± (57)	ND	+ (62)	**EF442949**	X83274
Desulforhabdus sp. strain BKA11	–	+ (58)	ND	+ (62)	**EF442950**	AJ012597
Desulforhabdus sp. strain DDT	–	± (58)	ND	+ (62)	**EF442951**	**EF442978**
Syntrophobacter fumaroxidans strain MPOB	10017^T	+‖		+‖	NZ_AAJ-F01000079	X82874

Table 1. cont.

Species*	Strain†	PCR product obtained with primer set‡			GenBank accession no.§	
		AprB-1-FW/ AprA-5-RV	AprA-1-FW/ AprA-10-RV	AprA-1-FW/ AprA-11-RV	*aprBA*	16S rRNA
Thermodesulforhabdus norvegica	9990^T	+ (54)	ND	+ (62)	**EF442952**	U25627
Phylum *Firmicutes*, *Clostridia*						
Peptococcaceae						
Desulfosporosinus orientis	765^T	± (54)	ND	− (50)	**EF442953**	Y11570
Desulfosporosinus meridei	13257^T	± (54)	ND	− (50)	**EF442954**	AF076527
Desulfotomaculum acetoxidans	771^T	+ (54)	− (50)	− (50)	**EF442955**	Y11566
Desulfotomaculum australicum	11792^T	+ (56)	ND	+ (62)	**EF442956**	M96665
Desulfotomaculum geothermicum	3669^T	− (50)	ND	+ (62)	**EF442957**	Y11567
Desulfotomaculum gibsoniae	7213^T	− (50)	ND	+ (62)	**EF442958**	AJ294431
Desulfotomaculum halophilum	11559^T	± (50)	ND	± (50)	**EF442959**	U88891
Desulfotomaculum luciae	12396^T	± (56)	ND	± (62)	**EF442960**	AF069293
Desulfotomaculum reducens strain MI-1	−	+‖		+‖	NZ_AAO-P01000042	U95951
Desulfotomaculum ruminis	2154^T	± (50)	ND	± (50)	**EF442961**	Y11572
Desulfotomaculum sapomandens	3223^T	− (50)	− (50)	+ (62)	**EF442962**	AF168365
Desulfotomaculum solfataricum	14956^T	± (54)	ND	+ (62)	**EF442963**	AY084078
Desulfotomaculum sp.	7440	± (50)	ND	± (50)	**EF442964**	Y11579
Desulfotomaculum sp.	7474	+ (58)	ND	+ (62)	**EF442965**	Y11577
Desulfotomaculum sp.	7475	+ (58)	ND	± (62)	**EF442966**	Y11580
Desulfotomaculum sp.	7476	+ (58)	ND	± (62)	**EF442967**	Y11578
Desulfotomaculum sp.	8775	+ (58)	ND	± (62)	**EF442968**	U33451
Desulfotomaculum thermoacetoxidans	5813^T	± (56)	ND	± (62)	**EF442969**	Y11573
Desulfotomaculum thermobenzoicum ssp. *thermobenzoicum*	6193^T	+ (58)	ND	± (62)	**EF442970**	Y11574
Desulfotomaculum thermobenzoicum ssp. *thermosyntrophicum*	14055^T	± (56)	ND	± (62)	**EF442971**	AY007190
Desulfotomaculum thermocisternum	10259^T	± (56)	ND	± (62)	**EF442972**	U33455
Desulfotomaculum thermosapovorans	6562^T	− (50)	ND	± (62)	**EF442973**	Y11575
Desulfotomaculum sp. strain JD-175	−	− (50)	ND	± (62)	**EF442975**	AF295656
Desulfotomaculum sp. strain JD-176	−	− (50)	ND	± (62)	**EF442976**	AF295657
Thermoanaerobacteriaceae						
Thermacetogenium phaeum	12270^T	+ (58)	+ (56)	− (50)	**EF442974**	AB020336

*Taxonomic classification of investigated SRP species is 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) and Kuever *et al.* (1999).

†DSMZ strain numbers; −, not deposited in a culture collection; T, type strain.

‡PCR amplification results with the respective primer pairs as follows: +, correct-sized PCR amplicon without byproducts; ±, correct-sized PCR amplicon with byproducts; −, no PCR amplicon obtained; ND, PCR amplification not determined. PCR annealing temperatures (°C) are in parentheses.

§*aprBA* and 16S rRNA gene sequences determined in this study are in bold type; NA, 16S rRNA gene sequence not available; SS, 16S rRNA gene sequence provided by S. Spring, DSMZ.

‖Binding of primer pair deduced from genomic *aprBA* sequence of respective SRP. +, Annealing possible; −, annealing inhibited (due to three or more mismatches).

terminal gene region, two reverse PCR primers of differing degeneracy (AprA-10-RV, AprA-11-RV) were developed that were complementary to the target site of either the *Desulfovibrio* species or the *Archaeoglobus*/Gram-positive SRB. The PCR primer pair AprB-1-FW/AprA-5-RV yielded a 1·2–1·35 kb *aprBA* amplicon from the investigated SRP, while an ~1·4 kb gene fragment of the 3′ terminal *aprA* gene region was amplified using the forward primer AprA-1-FW in combination with the reverse primers AprA-10-RV or AprA-11-RV. The *aprBA* and *aprA* PCR products overlap in sequence by ~400 bp (corresponding to *aprA* nucleotide positions 1236–1631 from *Desulfovibrio vulgaris*; Table 2).

PCR amplification of the *aprBA* gene fragments. PCR assays were performed with the RED*Taq* DNA Polymerase kit (Sigma-Aldrich). Reaction mixtures (50 µl total volume) contained 5 µl 10 × RED*Taq* PCR reaction buffer, 5 µl 10 × BSA solution (3 mg ml^{-1}), 200 µM dNTPs mixture, 1 µM each primer, 2·5 U RED*Taq* DNA Polymerase and 10–100 ng genomic DNA as template (water was

Table 2. PCR primers used for amplification of the *aprBA* and *aprA* gene fragments

Primer	Sequence (5′→3′)*	Primer binding site†
AprB-1-FW	TGC GTG TA**Y** AT**H** TG**Y** CC	272–288
AprA-1-FW	TGG CAG ATC ATG AT**Y** MA**Y** GG	1236–1256
AprA-5-RV	GCG CCA AC**Y** GG**R** CC**R** TA	1615–1631
AprA-10-RV	C**K**G **W**AG TAG **WA**R CCR GGR TA	2611–2630
AprA-11-RV	C**K**G **YR**R TAG TA**K** CC**S** GGC CA	2611–2630

*Degenerate positions are in bold type.
†Corresponding nucleotide positions of the *aprBA* operon of *Desulfovibrio vulgaris* ssp. *vulgaris* strain Hildenborough (GenBank accession no. Z69372).

used as a negative control in all PCR amplifications). The gene fragments were amplified in a PCR thermocycler (Eppendorf) by using a 'touchdown' PCR protocol. Thermal cycling was carried out as follows: 5 min of initial denaturation of DNA at 95 °C, followed by 35 cycles of denaturation at 95 °C for 60 s, a 'touchdown' annealing step for 90 s (annealing temperature was decreased in the first 20 cycles by 0·5 °C in every cycle, while the subsequent 15 cycles were carried out at constant temperature), and elongation at 72 °C for 120 s. Amplification was completed by a final elongation step at 72 °C for 10 min. The initial annealing temperature for the touchdown interval (10 °C) was altered in a range between 62 and 50 °C to optimize the amplification results with respect to the different templates. Aliquots of the amplicons (10 % of the reaction volume) were visually analysed by electrophoresis on 1 % (w/v) agarose gels run in 1 × Tris/borate/EDTA (TBE) buffer followed by ethidium bromide staining (0·5 mg ml^{-1}). Amplicons of the expected gene fragment size were purified using the QIAquick PCR purification kit, the QIAquick gel extraction kit (Qiagen) or the Perfectprep Gel Cleanup kit (Eppendorf) according to the suppliers' recommendations.

Cloning of PCR products. The 1·3 kb *aprBA* gene fragment of *Thermacetogenium phaeum* was ligated into pCR 2·1-TOPO vectors (TOPO TA cloning systems, Invitrogen) and transformed into chemically competent *Escherichia coli* TOP10 cells following the manufacturer's instructions. Clone plasmids with inserts of approximately 1·3 kb were selected and screened by PCR amplification with subsequent RFLP analysis of the amplicons. RFLP patterns were visualized on 2 % (w/v) agarose gel runs in 1 × TBE buffer and stained with ethidium bromide. Plasmids of representative clones from unique RFLP patterns were recovered with the QIAprep Spin kit (Qiagen).

Nucleotide sequencing. The PCR products (*aprBA*, *aprA* and 16S rDNA amplicons) were directly sequenced in both directions using the respective PCR amplification primers and the ABI BigDye Terminator Cycle Sequencing kit (Applied Biosystems). Sequencing reactions were run on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Sequence data analysis and phylogenetic tree inference. The DNA sequence data of the *aprBA* and *aprA* amplicons were assembled and manually corrected using the BioEdit (version 7.0.5) sequence alignment editor (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). BLAST searches for homologous sequences were performed at the NCBI web site (http://www.ncbi.nlm.nih.gov/BLAST/). Searches on the preliminary data of ongoing sequencing projects of SRP and SOB genomes were performed at The Institute for Genomic Research (TIGR) web site (http://www.tigr.org) and at the US Department of Energy (DOE) Joint Genome Institute web site (http://img.jgi.doe.gov/cgi-bin/pub/main.cgi). The deduced partial amino acid sequences of this study and the publicly available full-length AprBA sequences (summarized in Table 3) were automatically aligned using the web server Tcoffee@igs (http://igs-server.cnrs-mrs.fr/Tcoffee/). The frameshifted *aprB/A* sequences of the *Pyrobaculum aerophilum* genome were manually corrected before inclusion into the datasets. The nucleotide sequences were aligned according to the corrected amino acid alignment. The AprB and AprA datasets were analysed separately with the phylogeny inference methods included in the ARB software package (http://www.arb-home.de). Alignment regions of insertions and deletions (indels) were not considered in the phylogenetic analysis. Unrooted phylogenetic trees were calculated based on 103 (AprB) and 555/413 (AprA) amino acid positions using the ARB implemented program package (distance matrix, Fitch analysis; maximum-parsimony, ProtPars; maximum-likelihood, ProML and PUZZLE) and the PhyML program (maximum-likelihood method; http://atgc.lirmm.fr/phyml). Maximum-likelihood trees were constructed using the WAG or JTT amino acid substitution model matrices. The robustness of inferred trees was tested by bootstrap analysis with 100 resamplings. Tree reconstruction with PUZZLE analysis was performed by 20 000 Quartet Puzzling steps employing the WAG amino acid replacement model with either a unique rate of evolution or a mixed four-category discrete gamma heterogeneity model (approximation of parameters done using a neighbour-joining tree). Predictions of potential promoters, termination sites and operons in genome data were performed using the web versions FGENESB, BPROM and BTERM of the Softberry program package available at (http://www.softberry.com/berry.phtml). Protein secondary structure analysis and transmembrane helix prediction were done using the tools available at http://us.expasy.org/tools/#secondary.

The 16S rDNA sequences from the investigated reference strains (see Table 1 for GenBank accession nos) were loaded into the ARB database and aligned using the ARB_ALIGN tool. The maximum-parsimony method with a 50 % positional conservation filter and the Kimura two-parameter model of the ARB program package was used for phylogenetic tree calculations based on 1400 nucleotide positions. Partial 16S rDNA gene sequences were added to the calculated initial trees by applying the parsimony insertion tool of ARB without allowing changes in the overall tree topology. The robustness of the inferred tree was tested by bootstrap analysis with 100 resamplings using the PhyML program.

RESULTS

Amplification of *aprBA* genes by PCR from SRP

Using the PCR primer set AprB-1-FW/AprA-5-RV, we were able to amplify an *aprBA* gene fragment and establish a new *aprB* database from 95 distinct SRP species of 103 tested (Table 1). No *aprBA* amplicon was obtained from any of the six representatives of *Desulfotomaculum*

Table 3. Homologous genes coding for dissimilatory APS reductase and functionally associated proteins and their genetic arrangement in partial or complete genome sequences of prokaryotes

Str., strain.

SRP and SOB species	Homologous ORFs in genomic sequences (gene arrangement indicated by gene locus_tag no. annotation)						
	sat	*aprM*	*aprB*	*aprA*	*qmoA*	*qmoB*	*qmoC*
Chlorobia							
Chlorobaculum tepidum str. TLS	CT0862	–	CT0864	CT0865	CT0866	CT0867	CT0868
Chlorobium phaeobacteroides str. BS1	Cphamn1-DRAFT_1538	–	Cphamn1-DRAFT_1537	Incomplete ORF: *aprA*-NTCS in Cphamn1-DRAFT_1537 following sequence*	Incomplete ORF: *qmoA*-CTCS in Cphamn1-DRAFT_0878 preceding sequence*	Incomplete ORF: *qmoB*-NTCS in Cphamn1-DRAFT_0878*	Cphamn1-DRAFT_3474
Chlorobium chlorochromatium str. CaD3	Cag_1587	–	Cag_1586	Cag_1585	Cag_1584	Cag_1583	Cag_1582
Chlorobium clathratiforme str. BU-1	Ppha-DRAFT_2470	–	Ppha-DRAFT_2471	Ppha-DRAFT_2472	Ppha-DRAFT_2473	Ppha-DRAFT_2474	Ppha-DRAFT_2475
Alphaproteobacteria							
Uncultured bacterium EBAC2C11 (SAR116)	Red2C11_59	Red2C11_60	Red2C11_61	Red2C11_62	–	–	–
'*Candidatus* Pelagibacter ubique' HTCC1002 (SAR11)	–	PU1002_02221	PU1002_02216†	PU1002_02211	–	–	–
'*Candidatus* Pelagibacter ubique' HTCC1062 (SAR11)	–	SAR11_0835	SAR11_0836	SAR11_0837	–	–	–
Betaproteobacteria							
Thiobacillus denitrificans ATCC 25259	Tbd_0874	Tbd_2284	Tbd_0873/Tbd_2283	Tbd_0872/Tbd_2282	Tbd_1648	Tbd_1647	–
Gammaproteobacteria							
Allochromatium vinosum	AAC23622‡	AAK16200‡	AAC23620‡	AAC23621‡	–	–	–
Deltaproteobacteria							
Desulfotalea psychrophila str. LSv54	DP1472	–	DP1104	DP1105†	DP1106†	DP1107	DP1108
Desulfovibrio vulgaris ssp. *vulgaris* str. Hildenborough	DVU1295	–	DVU0846	DVU0847	DVU0848	DVU0849	DVU0850
Desulfovibrio desulfuricans str. G20	Dde_2265	–	Dde_1109	Dde_1110	Dde_1111	Dde_1112	Dde_1113
Syntrophobacter fumaroxidans str. MPOB	Sfum-DRAFT_3449	–	Incomplete ORF: *aprB*-NTCS in Sfum-DRAFT_3449 following sequence*	Incomplete ORF: *aprA*-CTCS in Sfum-DRAFT_3839*	Sfum-DRAFT_3840/Sfum-DRAFT_1034	Sfum-DRAFT_3841/Sfum-DRAFT_1035	Sfum-DRAFT_1036
Uncultured sulfate-reducing bacterium fosws39f7	39f70021	–	39f70022	39f70023	39f70001	39f70002	39f70020
Uncultured sulfate-reducing bacterium fosws7f8	ws7f8_12	–	ws7f8_11	ws7f8_10	–	–	ws7f8_3
Thermodesulfobacteriaceae							
Thermodesulfobacterium commune DSM 2178	TIGR contig 486	–	TIGR contig 486	TIGR contig 486	TIGR contig 486	TIGR contig 486	TIGR contig 486
Nitrospira							
Thermodesulfovibrio yellowstonii DSM 11347	TIGR contig 173	–	TIGR contig 173	TIGR contig 173	TIGR contig 173	TIGR contig 173	TIGR contig 173

Table 3. cont.

SRP and SOB species	Homologous ORFs in genomic sequences (gene arrangement indicated by gene locus_tag no. annotation)						
	sat	***aprM***	***aprB***	***aprA***	***qmoA***	***qmoB***	***qmoC***
Clostridia							
Desulfotomaculum reducens str. MI-1	Dred-DRAFT_3079		Dred-DRAFT_3080	Dred-DRAFT_3081	Dred-DRAFT_3082	Dred-DRAFT_3083	–
Euryarchaeota							
Archaeoglobus fulgidus DSM 4304	AF1667†	–	AF1669	AF1670	AF0663	AF0662	AF0661
Crenarchaeota							
Pyrobaculum aerophilum str. IM-2	PAE2609	–	PAE2561/PAE2562§	PAE2563/PAE2564§	–	–	–

*Abbreviations for incomplete ORF sequences received from draft genome projects: N- or C-terminal protein coding sequence (NTCS/CTCS).
†Incorrect ORF annotation by automated protein coding gene prediction program in genome sequence.
‡GenBank protein accession no. of respective ORF.
§Frameshift in sequence of respective ORF.

subcluster Ib (for *Desulfotomaculum* subcluster assignment, see Fig. 1), *Thermodesulforhabdus norvegica* or *Caldivirga maquilingensis*, while direct nucleotide sequencing of the *Desulfosporosinus orientis*, *Desulfobacterium niacini* and '*Desulfobacterium oleovorans*' strain HxD3 amplicon was unsuccessful with the reverse primer AprA-5-RV. The second primer set, AprA-1-FW combined with AprA-10-RV or AprA-11-RV, amplified the 3′-terminal *aprA* gene fragment from 93 out of 97 tested SRP species (Table 1). No *aprA* amplification product was obtained from the investigated *Desulfosporosinus* strains, *Desulfotomaculum acetoxidans* or *Caldivirga maquilingensis*. Direct nucleotide sequencing of the *Desulfotomaculum halophilum*, *Desulforhabdus amnigena*, *Desulfotalea psychrophila*, *Desulfobacterium catecholicum*, SRB strain Kette, *Desulfocella* sp. (DSM 2056) and *Thermacetogenium phaeum aprA* amplicon resulted in only partially evaluable sequences due to sequence ambiguities. The failure to amplify and sequence *apr* gene fragments from certain SRB species indicated that they do not contain target sites fully complementary to the applied primers. In fact, compositional analysis of the *aprBA* gene locus of *Desulfotalea psychrophila* (Rabus *et al.*, 2004) revealed a deviating codon usage that resulted in four mismatches at the primer binding site of Apr-10-RV. Nevertheless, the newly developed PCR primer sets enabled the enlargement of the present 0·9 kb *aprA* database (Friedrich, 2002) to a comprehensive *aprBA* database which comprises 2·2–2·4 kb (equivalent to 91–93 %) of the coding region of the dissimilatory APS reductase from 75 SRP species over a wide taxonomical range.

Molecular characterization of the *aprBA* gene locus in SRP

The sequence data from this study and the analysis of the available SRP and SOB genome data (Table 3) revealed a strictly conserved gene locus arrangement of *aprB* preceding the *aprA* gene and the absence of terminator-like signals downstream of the *aprB* stop codon, demonstrating that both genes form a transcriptional unit. The *aprBA* genes of SRP (and *Chlorobiaceae*) are generally separated by an intercistronic sequence region (16–148 nt) of a varying, family-specific length interval. As expected for highly expressed genes, the *aprA* genes from most investigated SRP possess Shine–Dalgarno (SD) sequences with entire complementarity (GGAGG) to the anti-SD core sequence and an optimal space distance between 6 and 12 nt (Kozak, 1999). AUG is used to start translation in all *aprA* sequences, with the exception of the members of *Desulfotomaculum* subcluster Ic, which utilize the less potent initiator GUG. The alternative start codon UUG (accession no. NC_006138, nucleotide position 1 246 432 in the genome sequence) annotated for the *aprA* gene of the *Desulfotalea psychrophila* genome (Rabus *et al.*, 2004) has to be corrected in our opinion to AUG (nucleotide position 1 246 438), which fulfils the required criteria (e.g. space distance of 9 nt instead of the annotated 3 nt to the SD sequence). In contrast, no intergenetic sequence exists in the *Allochromatium vinosum*-related sequence group as a consequence of the general overlapping of the *aprB* stop codon with the start codon of *aprA* in the sequence UGAUG or UAAUG.

Phylogeny of dissimilatory APS reductases from SRP

The AprB and AprA trees presented in this study were based on 110 AprB (Fig. 2) and 93 AprA sequences (Fig. 3), with 103 and 555 compared amino acid positions, respectively. AprA subtrees were calculated (413 compared positions) to include in the phylogenetic analysis partial sequences obtained from e.g. *Desulfotomaculum* subcluster

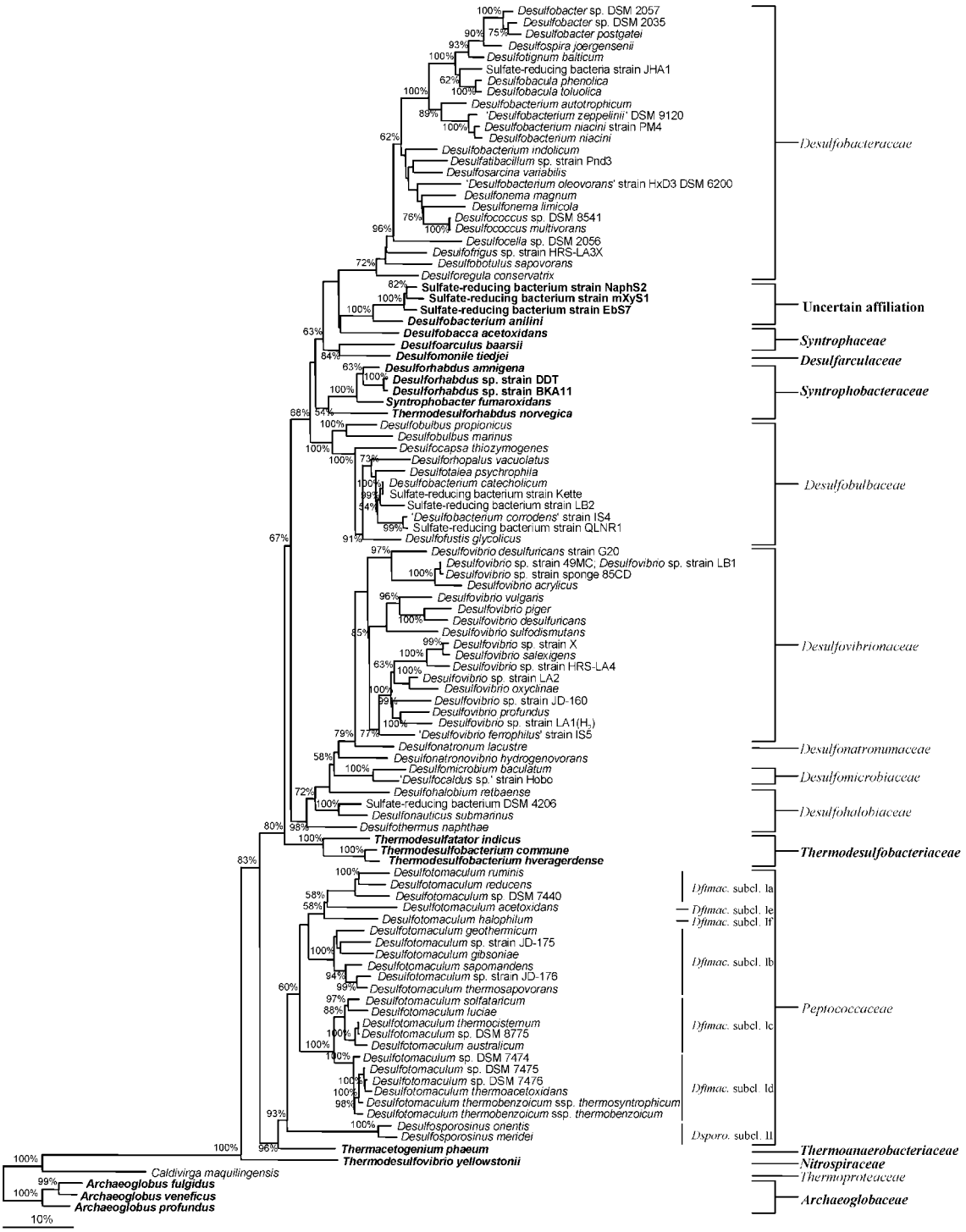

Fig. 1. Phylogenetic tree of 16S rRNA gene sequences from investigated SRP. The tree was constructed using the maximum-parsimony method with a 50 % positional conservation filter. The partial sequences of sulfate-reducing strains 49MC, LA1(H_2), LA2, LB1, sponge tissue 85CD and LB2 were inserted into the tree by applying parsimony criteria without allowing changes in the overall tree topology. *Archaeoglobus* species and *Caldivirga maquilingensis* were used as outgroup. The scale bar corresponds to 10 % estimated sequence divergence. Percentages greater than 50 % of bootstrap resampling that support each topological element are indicated near the nodes. SRP with laterally transferred *aprBA* genes are in bold type. Taxonomical classification of SRP into families and phylogenetic subclusters (subcl.) of Gram-positive SRB in the genera *Desulfotomaculum* (*Dftmac.*) and *Desulfosporosinus* (*Dsporo.*) (Kuever *et al.*, 1999) are indicated.

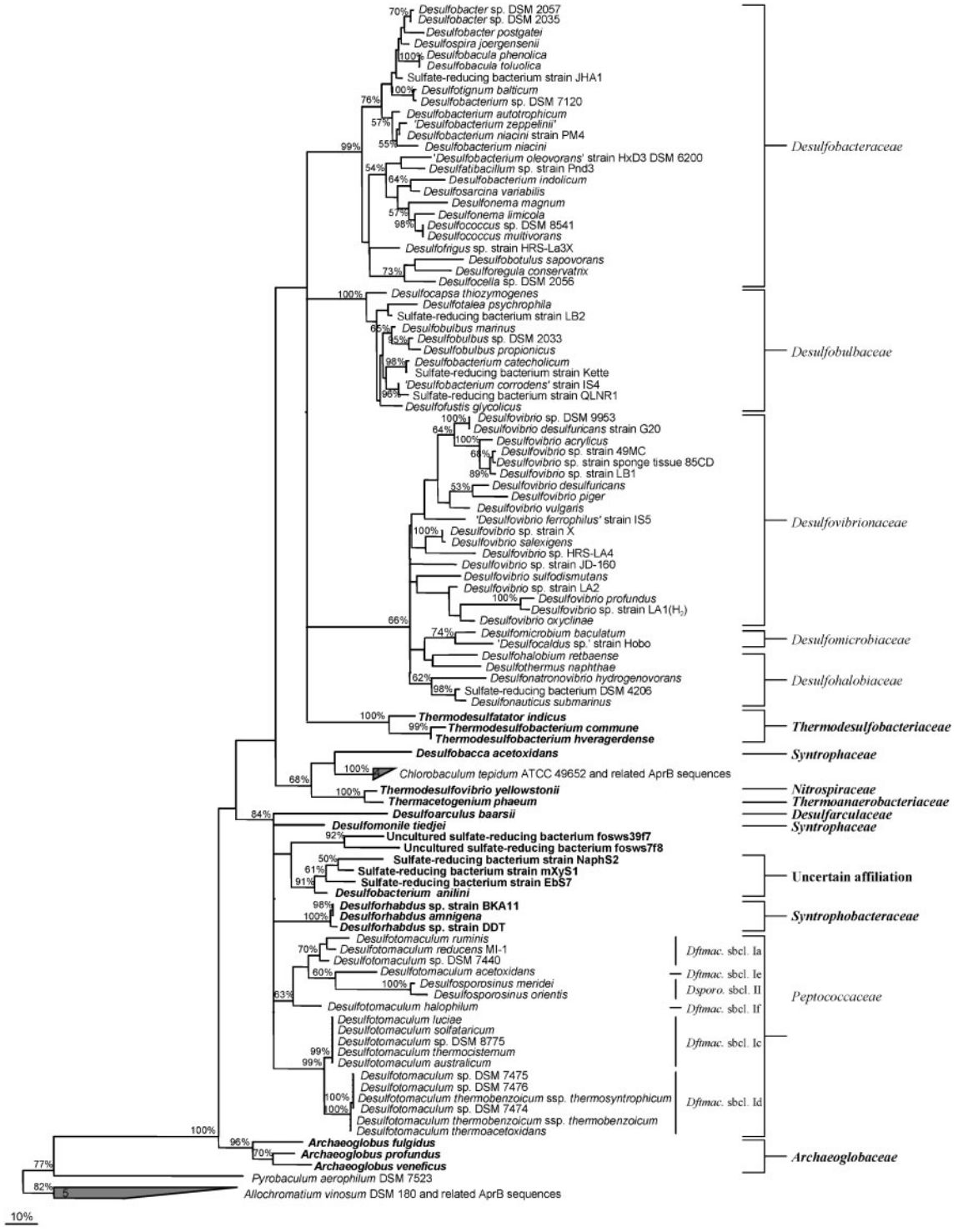

Fig. 2. Phylogenetic tree based on AprB sequences obtained from 92 investigated SRP including full-length protein sequences retrieved from public databases. The tree was inferred using PhyML (maximum-likelihood method). The *Allochromatium vinosum*-related sequences group and *Pyrobaculum aerophilum* were used as outgroup. The scale bar corresponds to 10 % estimated sequence divergence. Percentages greater than 50 % of bootstrap resampling that support each topological element are indicated near the nodes. SRP with laterally transferred *aprB* genes are in bold type. The consistent arrangements of SRP according to the 16S rRNA gene sequence-based taxonomical classification are indicated. Subcluster (sbcl.) assignment of the representatives of the genera *Desulfotomaculum* (*Dftmac.*) and *Desulfosporosinus* (*Dsporo.*) are according to Kuever *et al.* (1999).

Fig. 3. Phylogenetic tree based on AprA sequences obtained from 75 investigated SRP including full-length AprA sequences retrieved from public databases. The tree was inferred using PUZZLE analysis (maximum-likelihood method). The *Allochromatium vinosum*-related sequences group and *Pyrobaculum aerophilum* were used as outgroup. The scale bar corresponds to 10 % estimated sequence divergence. Reliability of branching order is indicated by numbers representing the quartet puzzling support values of the corresponding branches; polytomic nodes connect branches for which a relative order could not be determined unambiguously by PUZZLE. SRP with inferred laterally transferred *aprA* genes are in bold type. The 16S rRNA gene-based taxonomical classifications of the SRP species are indicated.

Ib (see Supplementary Fig. S1). The *Allochromatium vinosum*-related sequence group (including '*Candidatus* Pelagibacter ubique', uncultured bacterium EBAC2C11 and *Thiobacillus denitrificans*; see Table 3) and *Pyrobaculum aerophilum* were used to root the trees. The comparison of the AprB- and AprA-based tree topologies of the investigated SRP revealed a consistent separation into four distinct phylogenetic sequence clusters consisting of (1) *Archaeoglobus* species, (2) the Gram-positive SRB associated with members of the deltaproteobacterial *Syntrophobacterales*, *Desulfarcales* and the *Desulfobacterium anilini*-related SRB group, (3) the thermophilic SRB in affiliation with the *Chlorobaculum tepidum*-related sequences group (comprising *Chlorobium phaeobacteroides*, *Chlorobium chlorochromatium* and *Chlorobium clathratiforme*; see Table 3), and (4) the representatives of *Desulfovibrionales* and *Desulfobacterales* (Figs 2 and 3). The intracluster-branching order of taxa was consistent between the subunit trees, irrespective of the tree inference method or dataset used. However, topological discrepancies were obtained with respect to the relative ordering of the major SRP groups in the AprB and AprA trees, e.g. the varying position of the *Thermodesulfobacteriaceae* and *Archaeoglobus*. This might be explained by the limited amount of phylogenetic information contained in the smaller beta subunit, which is most likely insufficient to resolve branching orders at deeper phylogenetic levels. Nevertheless, the inferred phylogenies indicated the co-evolution of the beta and alpha subunits of the dissimilatory APS reductase in the investigated SRP.

Overall, the AprA trees presented in this work (Fig. 3, Supplementary Fig. S1) are topologically congruent with the tree of the earlier phylogenetic analysis which was based on 252 compared positions from AprA sequences of 60 SRP species and support the major LGT events postulated earlier (Friedrich, 2002). However, the AprA datasets used in the two phylogenetic studies overlap in only 30 investigated reference strains. The newly investigated genera and species, e.g. representatives of *Desulfosporosinus* and *Desulfotomaculum* subclusters Ic, Id and If (see Table 1), complete the previous Apr database for phylogenetic analyses and provide a more comprehensive framework for molecular ecology studies (Figs 2 and 3, Supplementary Fig. S1). The enlarged species coverage of this study refined and emphasized by novel results the general 16S rRNA gene congruence of the Apr-based inter- and intrafamily SRP relationships, e.g. the formation of the *Desulfovibrionaceae*, *Desulfonatronumaceae* and *Desulfomicrobiaceae* subclusters within the *Desulfovibrionales*. Interestingly, the Apr trees showed the *Desulfohalobiaceae* to be subdivided into two groups consisting of (1) *Desulfohalobium retbaense* related to the *Desulfomicrobiaceae*, and (2) *Desulfothermus naphthae*/*Desulfonatronovibrio hydrogenovorans*/*Desulfonauticus* sp. forming a basal-positioned group. The proposed 16S rRNA gene-based classification of the *Desulfotomaculum* and *Desulfosporosinus* members into the subclusters Ia–If and II (Kuever *et al.*, 1999) (Fig. 1) was supported by the Apr trees (Figs 2 and 3, Supplementary Fig. S1). In contrast with the earlier study (Friedrich, 2002), *Desulfarculus baarsii*, *Desulfomonile tiedjei* and the *Desulfobacterium anilini*-related SRB group branched separately within the Gram-positive AprB/A lineage. Furthermore, the family *Syntrophaceae* was not monophyletic, since *Desulfomonile tiedjei* formed a separate lineage within the *Desulfotomaculum* radiation, while the newly provided AprB/A sequences of *Desulfobacca acetoxidans* were by 16S rRNA gene-based phylogeny discordantly associated with the Gram-negative, thermophilic *Thermodesulfovibrio yellowstonii*. Another newly discovered deviation from the 16S rRNA gene-based phylogeny was the Apr-based, close relationship of the Gram-positive *Thermacetogenium phaeum* (Hattori *et al.*, 2000) with *Thermodesulfovibrio yellowstonii*. The cloning results of the *Thermacetogenium phaeum aprBA* amplicon demonstrated the absence of further gene copies in this reference strain. Direct nucleotide sequencing of its PCR-amplified 16S rDNA resulted in unambiguous nucleotide sequences. Indeed, the uncontaminated pure-culture status of the type strain of *Thermacetogenium phaeum* was also confirmed by the DSMZ (S. Spring, personal communication).

Additional evidence for lateral transfer of *aprBA* genes among SRP

The presence of indels at identical positions within the Apr sequences was used as additional evidence for the occurrence of the inferred LGT events. In addition, the new, enlarged *aprBA* sequence dataset was checked for recent LGTs by identification of (1) atypical *aprBA* sequence characteristics, such as significant G+C-content deviations (Lawrence & Ochman, 1997), and variations in codon usage with respect to the recipient genome and the *dsrAB* gene, and (2) sequence similarity of the *aprBA* intercistronic region between distantly related species. In this way, the separate phylogenetic position of the *Allochromatium vinosum*-related sequences group was confirmed by the presence of four unique indels in each subunit which were absent from AprB and AprA sequences of all SRP and the affiliated *Chlorobiaceae* cluster (see Supplementary Fig. S2 for amino acid sequence alignment). The proposed LGT of *aprBA* genes from Gram-positive SRB donor strains to the *Syntrophobacteraceae*, *Desulfomonile tiedjei* (including the unclassified putative SRB), the *Desulfobacterium anilini*-related SRB group, *Desulfarculus baarsii* as well as *Archaeoglobus* species were confirmed by the presence of six shared indels in the respective Apr sequences. The separate, basal branching point of the archaeal genus in AprB/A-based trees was supported by two additional unique indels. The close relationship of *Desulfobacca acetoxidans* to the *Thermodesulfovibrio–Chlorobaculum* cluster was confirmed by three unique indels located in the AprA sequence and the shared short C-terminal sequence of the AprB protein. No atypical sequence characteristics were detected in members

of the above recipient lineages that would indicate the recent occurrence of the proposed LGT events. However, the Apr-based close relationship of *Thermacetogenium phaeum* with *Thermodesulfovibrio* might be the first example of a recent LGT of *apr* genes among distantly related SRB reference strains. A recent lateral transfer of the *aprBA* gene to *Thermacetogenium phaeum* was supported by the presence of identical indel positions and lengths in the AprBA sequences of *Thermacetogenium phaeum* and *Thermodesulfovibrio* (see Supplementary Fig. S2), similar *aprBA* gene G+C content, codon usage, and the nearly identical length and nucleotide sequence of the intercistronic region.

Genomic arrangement of genes coding for the dissimilatory APS reductase and functionally associated proteins

The *sat*/*aprBA*/*qmoABC* gene organization of the available genomes reflected the phylogenetic divergence of the investigated SRP species into the four major AprBA lineages and their affiliation to the green sulfur bacteria (see Table 3 for gene locus numbers in genomes, and Fig. 4 for a graphical representation). The genomic arrangement in the thermophilic SRB, e.g. *Thermodesulfovibrio yellowstonii* and *Thermodesulfobacterium commune*, and the *Chlorobiaceae* was identical; the genes are most probably regulated in two separate operons, *sat-aprBA* and *qmoABC*. Notably, the *sat* gene is followed in all thermophilic SRP genomes by an additional ORF that encodes a protein of unknown function (*Archaeoglobus fulgidus*, AF1668; *Pyrobaculum aerophilum*, PAE2610). The *aprBA* and *qmoABC* gene arrangement in the genomes of the deltaproteobacterial representatives was similar to that of the thermophilic SRB and *Chlorobiaceae* genomes; however, the *sat* gene was separately located and regulated. Interestingly, the close relationship of the AprBA of *Syntrophobacter fumaroxidans* to the Gram-positive SRB lineage (e.g. *Desulfotomaculum reducens* MI-1) was reflected in their identical *sat-aprBA* and *qmoAB* operon structure; a sequence homologous to *qmoC* adjacent to this gene locus could not be identified in the preliminary genome data of these species. However, *Syntrophobacter fumaroxidans* harbours a second and functionally complete *qmoABC* operon that is not associated with the *sat-aprBA-qmoAB* gene cluster. A separately transcribed *qmoC* homologue is present near the *qmoAB* and *sat-aprBA* gene loci in the metagenomic sequences of the LGT-affected unclassified putative SRB strains (fosws39f7, fosws7f8) (Mussmann *et al.*, 2005). Interestingly, these strains are also closely related in the QmoA- and QmoB-based phylogenetic trees (not shown). This might indicate a concerted lateral transfer of the entire *sat-aprBA-qmoAB* gene cluster from the Gram-positive donor lineage (representative strain *Desulfotomaculum reducens*) to the deltaproteobacterial *Syntrophobacter fumaroxidans* and both uncultivated SRB strains (fosws39f7, fosws7f8), with subsequent intragenomic rearrangements (Suyama & Bork, 2001) in the uncultivated strains. Nevertheless, the presence of a (second) LGT-affected and separately located *qmoABC* gene locus in the *Syntrophobacter fumaroxidans* and *Archaeoglobus fulgidus* genomes demonstrates that independent lateral transfer of the *apr* and *qmo* genes has occurred.

The distant AprB/A-based phylogenetic relationship of the *Allochromatium vinosum*-related sequences group to the SRP and green sulfur bacteria is also reflected in the differing presence and genomic arrangement of genes that code for the functionally associated proteins. No *qmoABC*-homologous ORFs exist in the genomes of representatives of this sequence group (e.g. *Allochromatium vinosum*, '*Candidatus* Pelagibacter ubique'). Instead, the *aprBA* gene locus is always spatially associated and co-regulated with the unique gene *aprM* (encoding a putative membrane-bound protein), an arrangement that is not found in the genomes of SRP or *Chlorobiaceae*.

DISCUSSION

Phylogeny of the dissimilatory APS reductase from SRP

The consistent relative branching order of SRP taxa among the AprB and AprA trees indicates a shared evolutionary path for the *aprB* and *aprA* genes by vertical inheritance (speciation) and acquisition via concomitant LGT events, as demonstrated for the dissimilatory sulfite reductase-encoding genes *dsrA* and *dsrB* (Klein *et al.*, 2001; Molitor *et al.*, 1998; Zverlov *et al.*, 2005). The topology of the AprB/A-based trees of this study is congruent with the topology of an AprA-based tree of an earlier work (Friedrich, 2002). However, the two studies are based on different Apr datasets with respect to the SRP species coverage (102 versus 60 species) and the amount of phylogenetic information used for tree inference (2·2–2·4 kb versus 0·9 kb of the *apr* gene locus). The inferred phylogenetic positions of the Apr sequences from 72 newly investigated SRP species of this work were generally consistent with the major LGT events of *apr* genes proposed by Friedrich (2002) to have affected the sulfate-reducing members of the *Syntrophobacterales*, *Thermodesulfobacterium* and *Thermodesulfovibrio* species, as well as the archaeal genus *Archaeoglobus*. Nevertheless, the newly provided Apr sequences (Figs 2 and 3, Supplementary Fig. S1) refined and emphasized the 16S rRNA gene-congruent inter- and intrafamily clustering of the SRP taxa by the higher number of examined species. The results of this phylogenetic study prove the principal vertical transmission of the *aprBA* genes in the major SRP lineages. In contrast to the findings of the earlier analysis (Friedrich, 2002), the LGT-affected members of the *Syntrophobacteraceae*, the *Desulfobacterium anilini*-related SRB group, *Desulfomonile tiedjei* and *Desulfarculus baarsii* form four separately branching Apr lineages in affiliation with the monophyletic Gram-positive SRB group. Their separate phylogenetic placement

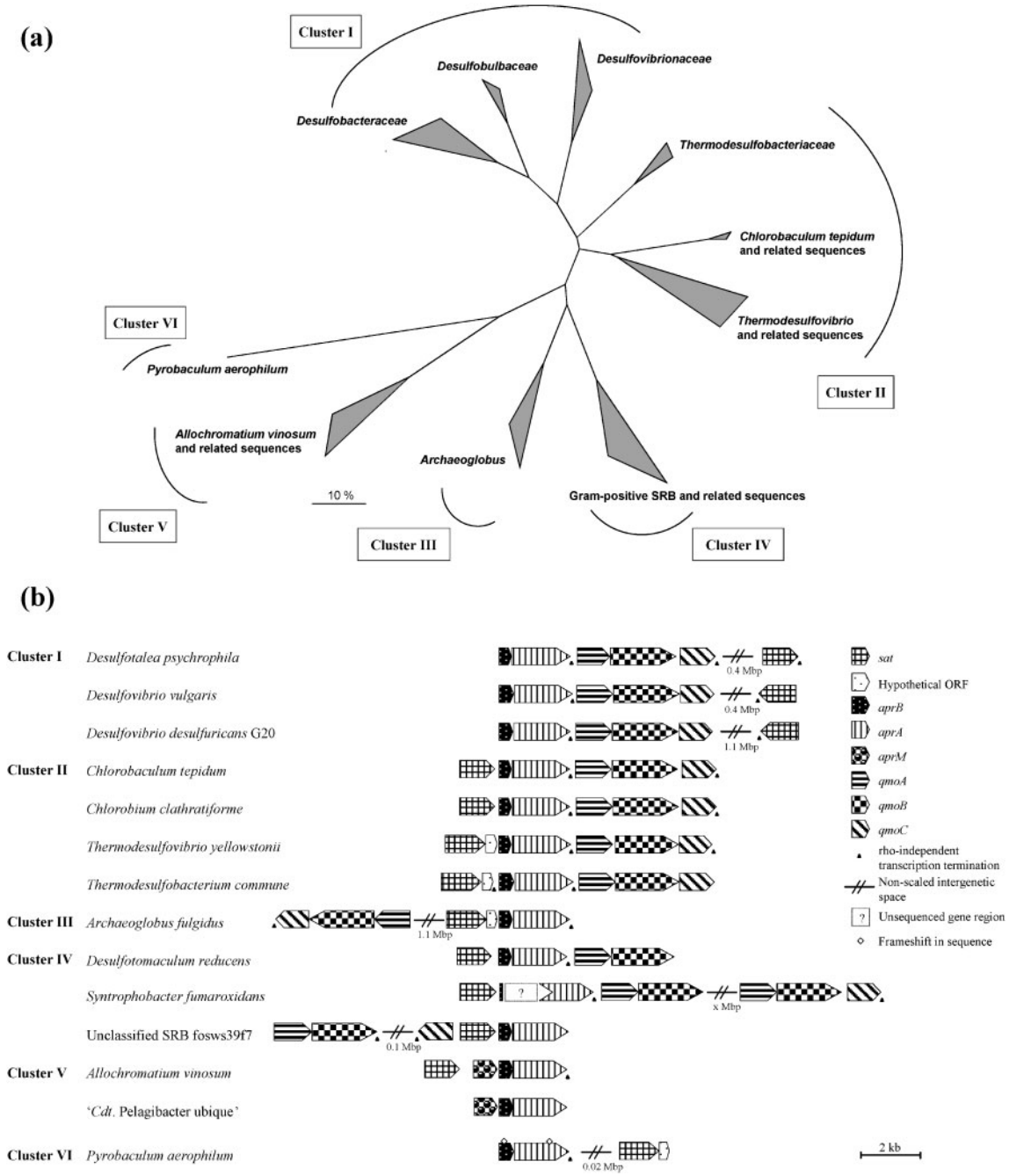

Fig. 4. (a) AprA-based phylogenetic tree of investigated SRP including full-length amino acid sequences of SRP and selected SOB received from public databases. The tree was inferred by Fitch analysis (distance matrix method). AprA sequences of SRP and SOB were graphically unified to their corresponding (16S rRNA gene-based) taxonomical groups of *Desulfovibrionales*, *Desulfobulbaceae*, *Desulfobacteraceae* (cluster I); *Thermodesulfobacteriaceae*, *Thermodesulfovibrio*, and *Chlorobaculum tepidum* and relatives (cluster II); *Archaeoglobus* (cluster III); Gram-positive SRB and LGT-affected deltaproteobacterial lineages (cluster IV); *Allochromatium vinosum*-related sequences group (cluster V); and *Pyrobaculum aerophilum* (cluster IV). The *Allochromatium vinosum*-related sequences group and *Pyrobaculum aerophilum* were used as outgroup. The scale bar corresponds to 10 % estimated sequence divergence. (b) Gene organization of the *sat*, *apr* and *qmo* loci (including predicted operon structure by Softberry) in sequenced SRP and SOB genomes and *Allochromatium vinosum*. ORFs were named based on BLASTX search results with significant homology scores. The following abbreviations were used for proposed homologous ORFs: *sat*, dissimilatory ATP sulfurylase; *aprA* and *aprB*, alpha and beta subunits of the dissimilatory APS reductase; *aprM*, putative transmembrane protein; *qmoA*, *qmoB* and *qmoC*, subunits of the putative menaquinone-oxidizing transmembrane protein complex. The cluster assignment refers to the enumeration of SRP and SOB in the AprA-based phylogenetic tree in (a).

points to four independent LGT events involving *apr* genes, most probably with different Gram-positive SRB species serving as donor strains. The different phylogenetic relationships of the xenologous *aprBA* genes from *Desulfomonile* and *Desulfobacca* indicate the incorrect systematic classification of both genera within the '*Syntrophaceae*', along with members that are not capable of sulfate or sulfite reduction (*Smithella* and *Syntrophus*) (Kuever *et al.*, 2005). Therefore, the AprB/A phylogenies support (1) the proposed taxonomical placement of *Desulfarculus baarsii* as well as *Desulfobacterium anilini* and its relatives into individual orders distinct from the *Syntrophobacterales* (Kuever *et al.*, 2005), and (2) the need for reclassification and validation of *Desulfomonile tiedjei* and *Desulfobacca acetoxidans* at the family level within the *Deltaproteobacteria*.

Two novel cases of LGT are proposed with respect to the xenologous Apr sequences of *Desulfobacca acetoxidans* and *Thermacetogenium phaeum* (also confirmed by indel pattern; see Supplementary Fig. S2). The *apr* gene composition analysis supported an ancient occurrence of the LGT event in *Desulfobacca acetoxidans*, whereas *Thermacetogenium phaeum* is the first reference strain reported to have been affected by a recent lateral transfer of *aprBA* genes. The type strain of *Thermacetogenium phaeum* was isolated from a thermophilic anaerobic methanogenic reactor (UASB), and to date is the only recognized sulfate-respiring strain within the Gram-positive *Thermoanaerobacteriales* (Hattori *et al.*, 2000). Its phylogenetic position in DsrAB-based trees has not yet been investigated and is therefore unknown. In support of a recent lateral *aprBA* transfer, the co-occurrence and predominance of *Thermacetogenium phaeum* and *Thermodesulfovibrio* species (recently classified as *Thermodesulfovibrio aggregans* and *Thermodesulfovibrio ethanolicus*; Y. Sekiguchi & H. Imachi, unpublished data) have been demonstrated by 16S rRNA analysis within the same granular sludge pellets of a thermophilic UASB reactor (Sekiguchi *et al.*, 1998). This close cell-to-cell contact would have increased the probability of interspecies LGT. The successful acquisition and functional implementation of this novel metabolic trait in the ancestor of *Thermacetogenium phaeum* might have been accelerated by certain genetic and physiological prerequisites: (1) the genomic arrangement (*sat-aprBA-qmoABC*) in the *Thermodesulfovibrio* donor strain (see genomic structure of *Thermodesulfovibrio yellowstonii*; Fig. 4b), which would have enabled a concomitant lateral transfer of all relevant genes in one single event; (2) the pre-existing capability for sulfite respiration in the ancestor of *Thermacetogenium phaeum* [see Supplementary Table S1 for the presence of *dsr* genes in the genomes of the related *Moorella thermoacetica* and *Carboxydothermus hydrogenoformans*; the capability of dissimilatory sulfite reduction has also been proven for *Carboxydothermus hydrogenoformans* (Henstra & Stams, 2004)]; and (3) the utilization of the same major membrane quinone component in the donor and recipient, menaquinone-7. The latter would have allowed an immediate linkage of the newly acquired sulfate-respiration system to the electron-transport processes in the ancestor of *Thermacetogenium phaeum*. Although the stable, specific conditions within the UASB reactor might have favoured the artificial generation of the SRB strain *Thermacetogenium phaeum*, it is a representative example of the permanently ongoing evolutionary process of gene flux among the genomes of free-living prokaryotes in nature (Daubin & Ochman, 2004; Jain *et al.*, 2003; Lerat *et al.*, 2005; Zhaxybayeva *et al.*, 2004).

Comparison of AprBA and DsrAB phylogeny from SRP

The topological comparison of AprB/A- and DsrAB-based trees revealed members of the same SRB lineages to be involved in lateral transfer of the *dsr* and *apr* genes. However, in the DsrAB-based tree, the deltaproteobacterial SRB group was monophyletic and the *Desulfobacterium anilini*-related SRB group was suggested to be the donor lineage for the xenologous *dsrAB* genes of the *Desulfotomaculum* subclusters Ib, Ic, Id and Ie, as well as *Moorella thermoacetica* (member of the *Thermoanaerobacteriales*). *Desulfosporosinus* and the *Desulfotomaculum* subclusters Ia, If and Ih have been postulated to represent the DsrAB-'authentic' Gram-positive SRB clades that were not affected by LGT (Imachi *et al.*, 2006; Klein *et al.*, 2001; Zverlov *et al.*, 2005). The xenologous gene displacements of the orthologous *dsrAB* genes in subclusters Ib–Ie are supported by the AprB/A phylogeny of this study, because *Desulfotomaculum* subclusters Ia/If and *Desulfosporosinus* consistently branch close to the root of the DsrAB- and AprB/A-based trees. According to the AprB/A phylogeny, the Gram-positive SRB species are monophyletic and present a relative branching order of taxa that is congruent with 16S rRNA and unaffected by LGT. Conversely, the acquisition of the *apr* gene of the deltaproteobacterial SRB lineages (*Syntrophobacteraceae*, the *Desulfobacterium anilini*-related SRB group, *Desulfomonile tiedjei* and *Desulfarculus baarsii*) postulated by four independent events of xenologous gene displacement is supported by the moderate relationships among their orthologous DsrAB sequences (Zverlov *et al.*, 2005). Indeed, this class of LGT has been reported to have influenced the evolutionary path of ~10–15 % of the orthologous genes in the bacterial domain (Novichkov *et al.*, 2004). The consistent xenologous branching positions from *Thermodesulfobacteriaceae* as well as *Archaeoglobus* in the AprA- and DsrAB-based trees (Klein *et al.*, 2001) point to a concerted acquisition of the novel capabilities to respire sulfite and sulfate by ancient, dual LGT events from unknown donor lineages; however, an early lateral transfer from Gram-positive SRB is suggested for the latter genus, since *Archaeoglobus* species seemed to be affiliated most closely with this lineage. In contrast, the ancestors of *Thermodesulfovibrio* might initially have been sulfite reducers, as indicated by the 16S rRNA-congruent branching of their DsrAB sequences (Klein *et al.*, 2001), and received their ability to respire

sulfate later on their evolutionary path. A donor lineage for the LGT of their *apr* genes is not apparent. Interestingly, a congruent taxonomical classification of both uncultivated putative SRB strains (fosws39f7, fosws7f8) (Mussmann *et al.*, 2005) was not possible on the basis of the Dsr and Apr sequences. A distinct origin is proposed for their xenologous *apr* and *dsr* genes, which are arranged as a metabolic island.

Correlation between Apr phylogeny and presence of proteins functionally associated with AprBA (Qmo and AprM) in SRP and SOB

The genome analysis indicated the development of two unrelated protein (complexes), AprM and QmoABC, mediating the electron transfer between the cytoplasmic sulfite oxidation/APS reduction process and the membrane quinone pool in correlation with the Apr phylogeny-based divergence of the SRP and SOB. The membrane-bound redox complex QmoABC that has been investigated and characterized from *Desulfovibrio desulfuricans* ATCC 27774 is proposed to act as a menaquinol/APS reductase oxidoreductase (Pires *et al.*, 2003). In support, further experimental studies have demonstrated the coordinated down-regulation of the *apr* and *qmo* gene sets in a *Desulfovibrio vulgaris* strain Hildenborough mutant in the presence of nitrite (Haveman *et al.*, 2004), and the dependence of the sulfate-reduction capability of *Desulfotomaculum aeronauticum* on amendment with the quinone precursor menadione (Hippe *et al.*, 1997). Indeed, (1) the presence of the *qmoABC* genes and their close proximity to the *aprBA* genes in all genomes of SRP (see Table 3, Fig. 4) (whereas they are absent in sulfite- or thiosulfate-reducing species which lack the *aprBA* genes; Supplementary Table S1), and (2) the congruent protein phylogenies (trees not shown), support the proposed functional association of the Qmo complex and the APS reductase in SRP. The presence of SRP-related *apr* and *qmo* genes in *Chlorobiaceae* genomes is an indication of an electron transfer system similar to that of SRP in the green sulfur bacteria. In contrast, in genomes of SOB harbouring *Allochromatium vinosum*-related Apr sequences, the *aprBA* genes are always co-regulated with a preceding *aprM* (encoding a membrane-integral protein), whereas ORFs homologous to *qmoABC* are absent (see Table 3, Fig. 4b). The strictly conserved arrangement and the AprBA-congruent AprM tree topology (not shown) point to an essential role for AprM in *Allochromatium vinosum* and other Apr-related SOB. An *in vivo* function as a structural component (membrane anchor) for the cytoplasmic APS reductase or even a direct functional involvement in electron transfer, by analogy with the menaquinol : fumarate oxidoreductase of *E. coli* (Cecchini *et al.*, 2002; Lancaster, 2003), are possible.

Unlike the APS reductase, the transmembrane redox complex that is functionally associated with the dissimilatory sulfite reductase (DsrAB) seems to be identical among the SRP and SOB. The homologous HmeABCDE and DsrMKJOP protein complexes have been proposed to operate in the electron transfer pathway between cytoplasmic DsrAB and the membrane-integral quinol/quinone pool (Dahl *et al.*, 2005; Mander *et al.*, 2002; Pires *et al.*, 2006; Sander *et al.*, 2006). The genome analyses in this study revealed DsrMKJOP homologues to be present in all sulfate- and sulfite-reducing as well as several sulfur-oxidizing bacteria (Supplementary Table S1).

Evolutionary scenario for the dissimilatory APS reductase as a key enzyme of the sulfate-reduction pathway in the light of Apr and Dsr phylogeny and geochemical data of Archean Earth

The global appearance of mass-independent isotope fractionation (MIF) in sedimentary sulfides and sulfates older than 2·45 billion years is consistent with low oxygen levels in the atmosphere ($pO_2 < 10^{-5}$ present atmospheric level; PAL) and a global sulfur cycle dominated by atmospheric reactions during the Archean era (4·0–2·4 billion years ago) (Farquhar *et al.*, 2000). Atmospheric sulfate generated by photolysis of SO_2 would have represented the only significant abiogenic sulfate load of the ocean (Canfield, 2005; Farquhar *et al.*, 2000; Strauss, 2003). Consistently, the absence of significant mass-dependent isotope fractionation between Archean sulfate and sulfide is an indication of a global sulfate concentration below 200 μM (Canfield *et al.*, 2000; Canfield, 2001; Habicht *et al.*, 2002) and the absence of an active global sulfur cycle in the Archean hydrosphere (Strauss, 2003). Sulfate in concentrations sufficient for energy conservation by sulfate respiration could have only been supplied by the metabolic activity of sulfur-oxidizing anoxygenic phototrophic bacteria (Canfield & Raiswell, 1999; Shen *et al.*, 2001). Indeed, photosynthetic microbial mats dominated by anoxygenic phototrophic bacteria existed at 3·4 billion years ago (Tice & Lowe, 2004). The ancestors of the modern SRP could have originated in dependence on the favourable conditions provided by the sulfur-oxidizing anoxygenic phototrophic bacteria, and the sulfate-reduction process might have become established and geochemically expressed as early as 3·4 billion years ago (Shen *et al.*, 2001) at local, restricted sites.

In agreement with the proposed geochemical setting of the Archean Earth, the Dsr and Apr phylogenies (Boucher *et al.*, 2003; this study) consistently point to their origin and evolution as oxidative-operating enzymes. Since the DsrAB has been suggested to be of ancient origin (Dhillon *et al.*, 2005), the reverse sulfate-reduction pathway might have evolved successively (sulfite reductase preceding the APS reductase and ATP sulfurylase) in an ancestral anoxygenic phototrophic bacterium. The development of DsrAB and its functionally associated DsrMKJOP complex (Dahl *et al.*, 2005; Mander *et al.*, 2002; Sander *et al.*, 2006) would have allowed the utilization of hydrothermal-derived sulfide as a

reductant for anoxygenic photosynthesis. The subsequent phylogenetic divergence into three DsrAB lineages (Boucher *et al.*, 2003) might have been the result of two early, independent LGTs of the progenotic *dsrAB* (in concert with the *dsrMKJOP* genes) from the ancestral sulfide-oxidizing bacterial donor lineage to the ancestors of SRP and sulfur-respiring archaeal *Pyrobaculum*. In the same way as their modern equivalents, the ancient microbial mats might have presented hot spots for the metabolic diversification of the microbial community by LGT (Molin & Tolker-Nielsen, 2003; Sorensen *et al.*, 2005). The ancestors of the SRB lineages might initially have been sulfite respirers by adaptive reversal of the oxidative-operating Dsr protein sets. The SRP-related DsrMKJOP of the *Chlorobiaceae* (Sander *et al.*, 2006) might have arisen from an early xenologous replacement of the *dsrMKJOP* genes of an ancestral green sulfur bacterium with those from a sulfite reducer.

Since the end product of Dsr-mediated sulfide/sulfur oxidation is sulfite, the reverse APS reductase might primarily have been developed by the ancestral sulfide oxidizers for detoxification instead of energy conservation. Indeed, a sulfite-oxidation pathway via the intermediate APS would have allowed the simultaneous generation of energy (ATP) by substrate phosphorylation, which would have contributed to relieving energy limitation in a primitive anoxygenic phototrophic bacterium. Two conflicting scenarios would explain the DsrAB-incongruent AprBA tree topology and the appearance of two different membrane protein(s) systems that interact with APS reductase. First, after phylogenetic separation of the progenotic detoxifying APS reductase into the lineages of the anoxygenic phototrophic green and purple sulfur bacteria, the protein sets QmoABC and AprM emerged independently in these groups and allowed the utilization of sulfite as a reductant in anoxygenic photosynthesis. The concurrent presence of *apr* and *qmo* genes in *Chlorobiaceae* and SRP genomes would have been the result of a subsequent, concerted LGT from an ancestral green sulfur bacterial donor to the sulfite-respiring ancestors of the SRP lineages. The second, alternative scenario implies an early divergence of the progenotic detoxifying AprBA into the phylogenetic lineages of the SOB and the SRP analogous to the postulated evolutionary path of DsrAB (Boucher *et al.*, 2003; Molitor *et al.*, 1998). The Qmo complex would then have originated within an ancestral sulfite-reducing bacterial lineage and not in an ancestor of the green sulfur bacteria, whereas AprM developed in ancestral purple sulfur bacteria. Because of the restricted distribution of the *apr* and *qmo* genes within the *Chlorobiaceae* (B. Meyer and J. Kuever, unpublished results), their ancestors either never possessed or lost their ancestral *apr* genes due to functional replacement by convergently evolved proteins. Indeed, a thermophilic sulfate-reducing lineage (e.g. *Thermodesulfovibrio*) could have served as donor for the later acquisition or reacquisition of the entire gene locus by LGT. Irrespective of possible evolutionary scenarios, the basal branching AprBA lineage of *Pyrobaculum aerophilum* might represent the APS reductase type most closely related to the progenotic detoxifying form, because no gene coding for any of the proposed functionally associated proteins is present in the genome. The patchy and polyphyletic distribution of the sulfate-reduction pathway among prokaryotes and the late diversification of the major SRP lineages, despite the postulated early origin of the respiration process (Shen *et al.*, 2001), might be the result of the persistently low sulfate content of ocean waters until 2·4 billion years ago (Canfield *et al.*, 2000; Canfield, 2005; Farquhar *et al.*, 2000; Habicht *et al.*, 2002; Strauss, 2003), which restricted the abundance and ecological significance of this physiological group in the Archean era. The radiation of the SRP might have started with the beginning of the oxygenation of the atmosphere (Canfield *et al.*, 2000; Canfield, 2005; Farquhar *et al.*, 2000), which resulted in an increasing oceanic sulfate concentration during the Proterozoic era (2·5–0·54 billion years ago) (Canfield, 2005; Kah *et al.*, 2004). A widespread lateral distribution of the sulfate-reduction pathway via mobilizable metabolic islands has been suggested (Friedrich, 2002; Mussmann *et al.*, 2005). However, (1) the absence of characteristic mobility elements indicative of classical genomic islands in the metagenome sequences (Mussmann *et al.*, 2005), (2) the generally scattered arrangement of the *dsr* and *apr* genes in the genomes of validated SRP, and (3) the differing phylogenies of DsrAB (Boucher *et al.*, 2003; Klein *et al.*, 2001; Zverlov *et al.*, 2005) compared with those of AprBA (this study) and Sat (Sperling *et al.*, 1998) caused by non-parallel LGT events, seem to contradict this hypothesis for the evolution of the dissimilatory sulfate-reduction pathway.

NOTE ADDED IN PROOF

While this paper was under review, *sat*, *aprBA* and *qmoABC* gene sequences of further SRB were made available in public databases by the (meta)genome sequencing projects of *Desulfovibrio vulgaris* strain DP4 (NC_008751), deltaproteobacterium strain MLMS-1 (NZ_AAFQ01000037, NZ_AAFQ01000064 and NZ_AAFQ01000396) and *Desulfosarcina/Desulfonema*-related symbionts of *Olavius algarvensis* (AASZ_01000000). The gene arrangements in the genomes of these SRB are identical to those of *Desulfovibrio* spp. and *Desulfotalea psychrophila* as presented in this work.

ACKNOWLEDGEMENTS

This study was supported by grants of the Bundesministerium für Bildung und Forschung (BMBF) (project 'Caribflux' under contract no. 03G0154C), the Deutsche Forschungsgemeinschaft (DFG) (under contract no. KU 916/8-1) and the Max Planck Society, Munich.

REFERENCES

Boucher, Y., Douady, C. J., Papke, R. T., Walsh, D. A., Boudreau, M. E. R., Nesbo, C. L., Case, R. J. & Doolittle, W. F. (2003). Lateral gene

transfer and the origins of prokaryotic groups. *Annu Rev Genet* **37**, 283–328.

Canfield, D. E. (2001). Isotope fractionation by natural populations of sulfate-reducing bacteria. *Geochim Cosmochim Acta* **65**, 1117–1124.

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. & Raiswell, R. (1999). The evolution of the sulfur cycle. *Am J Sci* **299**, 697–723.

Canfield, D. E., Habicht, K. S. & Thamdrup, B. (2000). The Archean sulfur cycle and the early history of atmospheric oxygen. *Science* **288**, 658–661.

Cecchini, G., Schröder, I., Gunsalus, R. P. & Maklashina, E. (2002). Succinate dehydrogenase and fumarate reductase from *Escherichia coli*. *Biochim Biophys Acta* **1553**, 140–157.

Dahl, C. & Trüper, H. G. (1994). Enzymes of dissimilatory sulfide oxidation in phototrophic sulfur bacteria. *Methods Enzymol* **243**, 400–421.

Dahl, C., Engels, S., Pott-Sperling, A. S., Schulte, A., Sander, J., Lubbe, Y., Deuster, O. & Brune, D. C. (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.

Daubin, V. & Ochman, H. (2004). Bacterial genomes as new gene homes: the genealogy of ORFans in *E. coli*. *Genome Res* **14**, 1036–1042.

DeLong, E. F. (1992). Archaea in coastal marine environments. *Proc Natl Acad Sci U S A* **89**, 5685–5689.

Dhillon, A., Goswami, S., Riley, M., Teske, A. & Sogin, M. (2005). Domain evolution and functional diversification of sulfite reductases. *Astrobiology* **5**, 18–29.

Farquhar, J., Bao, H. M. & Thiemens, M. (2000). Atmospheric influence of Earth's earliest sulfur cycle. *Science* **289**, 756–758.

Friedrich, M. W. (2002). Phylogenetic analysis reveals multiple lateral transfers of adenosine-5′-phosphosulfate reductase genes among sulfate-reducing microorganisms. *J Bacteriol* **184**, 278–289.

Fritz, G., Buchert, T., Huber, H., Stetter, K. O. & Kroneck, P. M. H. (2000). Adenylylsulfate reductases from archaea and bacteria are 1 : 1 alpha beta-heterodimeric iron-sulfur flavoenzymes – high similarity of molecular properties emphasizes their central role in sulfur metabolism. *FEBS Lett* **473**, 63–66.

Gogarten, J. P. & Townsend, J. P. (2005). Horizontal gene transfer, genome innovation and evolution. *Nat Rev Microbiol* **3**, 679–687.

Habicht, K. S., Gade, M., Thamdrup, B., Berg, P. & Canfield, D. E. (2002). Calibration of sulfate levels in the Archean ocean. *Science* **298**, 2372–2374.

Hattori, S., Kamagata, Y., Hanada, S. & Shoun, H. (2000). *Thermacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. *Int J Syst Evol Microbiol* **50**, 1601–1609.

Haveman, S. A., Greene, E. A., Stilwell, C. P., Voordouw, J. K. & Voordouw, G. (2004). Physiological and gene expression analysis of inhibition of *Desulfovibrio vulgaris* Hildenborough by nitrite. *J Bacteriol* **186**, 7944–7950.

Henstra, A. M. & Stams, A. J. M. (2004). Novel physiological features of *Carboxydothermus hydrogenoformans* and *Thermoterrabacterium ferrireducens*. *Appl Environ Microbiol* **70**, 7236–7240.

Hipp, W. M., Pott, A. S., Thum-Schmitz, N., Faath, I., Dahl, C. & Trüper, H. G. (1997). Towards the phylogeny of APS reductases and sirohaem sulfite reductases in sulfate-reducing and sulfur-oxidizing prokaryotes. *Microbiology* **143**, 2891–2902.

Hippe, H., Hagenauer, A. & Kroppenstedt, R. M. (1997). Menadione requirement for sulfate-reduction in *Desulfotomaculum aeronauticum*, and emended species description. *Syst Appl Microbiol* **20**, 554–558.

Imachi, H., Sekiguchi, Y., Kamagata, Y., Loy, A., Qiu, Y. L., Hugenholtz, P., Kimura, N., Wagner, M., Ohashi, A. & Harada, H. (2006). Non-sulfate-reducing, syntrophic bacteria affiliated with *Desulfotomaculum* cluster I are widely distributed in methanogenic environments. *Appl Environ Microbiol* **72**, 2080–2091.

Itoh, T., Suzuki, K., Sanchez, P. C. & Nakase, T. (1999). *Caldivirga maquilingensis* gen. nov., sp. nov., a new genus of rod-shaped crenarchaeote isolated from a hot spring in the Philippines. *Int J Syst Bacteriol* **49**, 1157–1163.

Jain, R., Rivera, M. C., Moore, J. E. & Lake, J. A. (2003). Horizontal gene transfer accelerates genome innovation and evolution. *Mol Biol Evol* **20**, 1598–1602.

Kah, L. C., Lyons, T. W. & Frank, T. D. (2004). Low marine sulphate and protracted oxygenation of the proterozoic biosphere. *Nature* **431**, 834–838.

Klein, M., Friedrich, M., Roger, A. J., Hugenholtz, P., Fishbain, S., Abicht, H., Blackall, L. L., Stahl, D. A. & Wagner, M. (2001). Multiple lateral transfers of dissimilatory sulfite reductase genes between major lineages of sulfate-reducing prokaryotes. *J Bacteriol* **183**, 6028–6035.

Kozak, M. (1999). Initiation of translation in prokaryotes and eukaryotes. *Gene* **234**, 187–208.

Kuever, J., Rainey, F. A. & Hippe, H. (1999). Description of *Desulfotomaculum* sp. Groll as *Desulfotomaculum gibsoniae* sp. nov. *Int J Syst Bacteriol* **49**, 1801–1808.

Kuever, J., Rainey, F. A. & Widdel, F. (2005). The deltaproteobacterial orders *Desulfovibrionales, Desulfobacterales, Desulfarcales* and *Syntrophobacterales*. In *Bergey's Manual of Systematic Bacteriology*, pp. 925–1040. Edited by G. Garrity. New York: Springer.

Kurland, C. G., Canback, B. & Berg, O. G. (2003). Horizontal gene transfer: a critical view. *Proc Natl Acad Sci U S A* **100**, 9658–9662.

Lampreia, J., Pereira, A. S. & Moura, J. J. G. (1994). Adenylylsulfate reductases from sulfate-reducing bacteria. *Methods Enzymol* **243**, 241–260.

Lancaster, C. R. D. (2003). *Wolinella succinogenes* quinol : fumarate reductase and its comparison to *E. coli* succinate : quinone reductase. *FEBS Lett* **555**, 21–28.

Lawrence, J. G. & Ochman, H. (1997). Amelioration of bacterial genomes: rates of change and exchange. *J Mol Evol* **44**, 383–397.

Lerat, E., Daubin, V., Ochman, H. & Moran, N. A. (2005). Evolutionary origins of genomic repertoires in bacteria. *PLoS Biol* **3**, e130.

Mander, G. J., Duin, E. C., Linder, D., Stetter, K. O. & Hedderich, R. (2002). Purification and characterization of a membrane-bound enzyme complex from the sulfate-reducing archaeon *Archaeoglobus fulgidus* related to heterodisulfide reductase from methanogenic archaea. *Eur J Biochem* **269**, 1895–1904.

Molin, S. & Tolker-Nielsen, T. (2003). Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure. *Curr Opin Biotechnol* **14**, 255–261.

Molitor, M., Dahl, C., Molitor, I., Schafer, U., Speich, N., Huber, R., Deutzmann, R. & Trüper, H. G. (1998). A dissimilatory sirohaem-sulfite-reductase-type protein from the hyperthermophilic archaeon *Pyrobaculum islandicum*. *Microbiology* **144**, 529–541.

Mori, K., Kim, H., Kakegawa, T. & Hanada, S. (2003). A novel lineage of sulfate-reducing microorganisms: *Thermodesulfobiaceae* fam. nov., *Thermodesulfobium narugense*, gen. nov., sp. nov., a new thermophilic isolate from a hot spring. *Extremophiles* **7**, 283–290.

Moussard, H., L'Haridon, S., Tindall, B. J., Banta, A., Schumann, P., Stackebrandt, E., Reysenbach, A. L. & Jeanthon, C. (2004).

Thermodesulfatator indicus gen. nov., sp. nov., a novel thermophilic chemolithoautotrophic sulfate-reducing bacterium isolated from the Central Indian Ridge. *Int J Syst Evol Microbiol* **54**, 227–233.

Mussmann, M., Richter, M., Lombardot, T., Meyerdierks, A., Kuever, J., Kube, M., Glockner, F. O. & Amann, R. (2005). Clustered genes related to sulfate respiration in uncultured prokaryotes support the theory of their concomitant horizontal transfer. *J Bacteriol* **187**, 7126–7137.

Muyzer, G., Teske, A., Wirsen, C. O. & 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.

Novichkov, P. S., Omelchenko, M. V., Gelfand, M. S., Mironov, A. A., Wolf, Y. I. & Koonin, E. V. (2004). Genome-wide molecular clock and horizontal gene transfer in bacterial evolution. *J Bacteriol* **186**, 6575–6585.

Pires, R. H., Lourenco, A. I., Morais, F., Teixeira, M., Xavier, A. V., Saraiva, L. M. & Pereira, I. A. C. (2003). A novel membrane-bound respiratory complex from *Desulfovibrio desulfuricans* ATCC 27774. *BBA Bioenergetics* **1605**, 67–82.

Pires, R. H., Venceslau, S. S., Morais, F., Teixeira, M., Xavier, A. V. & Pereira, I. A. C. (2006). Characterization of the *Desulfovibrio desulfuricans* ATCC 27774 DsrMKJOP complex – a membrane-bound redox complex involved in the sulfate respiratory pathway. *Biochemistry* **45**, 249–262.

Rabus, R., Hansen, T. A. & Widdel, F. (1999). Dissimilatory sulfate- and sulfur-reducing prokaryotes. In *The Prokaryotes: an Evolving Electronic Database for the Microbiological Community*, pp. 1–87. Edited by M. Dworkin, K.-H. Schleifer & E. Stackebrandt. New York: Springer.

Rabus, R., Ruepp, A., Frickey, T., Rattei, T., Fartmann, B., Stark, M., Bauer, M., Zibat, A., Lombardot, T. & other authors (2004). The genome of *Desulfotalea psychrophila*, a sulfate-reducing bacterium from permanently cold Arctic sediments. *Environ Microbiol* **6**, 887–902.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sander, J., Engels-Schwarzlose, S. & Dahl, C. (2006). Importance of the DsrMKJOP complex for sulfur oxidation in *Allochromatium vinosum* and phylogenetic analysis of related complexes in other prokaryotes. *Arch Microbiol* **186**, 357–366.

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

Schedel, M. & Trüper, H. G. (1980). Anaerobic oxidation of thiosulfate and elemental sulfur in *Thiobacillus denitrificans*. *Arch Microbiol* **124**, 205–210.

Sekiguchi, Y., Kamagata, Y., Syutsubo, K., Ohashi, A., Harada, H. & Nakamura, K. (1998). Phylogenetic diversity of mesophilic and thermophilic granular sludges determined by 16S rRNA analysis. *Microbiology* **144**, 2655–2665.

Shen, Y. A., Buick, R. & Canfield, D. E. (2001). Isotopic evidence for microbial sulphate reduction in the early Archaean era. *Nature* **410**, 77–81.

Sorensen, S. J., Bailey, M., Hansen, L. H., Kroer, N. & Wuertz, S. (2005). Studying plasmid horizontal transfer in situ: a critical review. *Nat Rev Microbiol* **3**, 700–710.

Speich, N., Dahl, C., Heisig, P., Klein, A., Lottspeich, F., Stetter, K. O. & Trüper, H. G. (1994). Adenylylsulphate reductase from the sulfate-reducing archaeon *Archaeoglobus fulgidus* – cloning and characterization of the genes and comparison of the enzyme with other iron-sulfur flavoproteins. *Microbiology* **140**, 1273–1284.

Sperling, D., Kappler, U., Wynen, A., Dahl, C. & Trüper, H. G. (1998). Dissimilatory ATP sulfurylase from the hyperthermophilic sulfate reducer *Archaeoglobus fulgidus* belongs to the group of homo-oligomeric ATP sulfurylases. *FEMS Microbiol Lett* **162**, 257–264.

Strauss, H. (2003). Sulphur isotopes and the early Archaean sulphur cycle. *Precambrian Res* **126**, 349–361.

Suyama, M. & Bork, P. (2001). Evolution of prokaryotic gene order: genome rearrangements in closely related species. *Trends Genet* **17**, 10–13.

Tice, M. M. & Lowe, D. R. (2004). Photosynthetic microbial mats in the 3,416-Myr-old ocean. *Nature* **431**, 549–552.

Trüper, H. G. & Fischer, U. (1982). Anaerobic oxidation of sulfur-compounds as electron-donors for bacterial photosynthesis. *Philos Trans R Soc Lond B Biol Sci* **298**, 529–542.

Zhaxybayeva, O., Lapierre, P. & Gogarten, J. P. (2004). Genome mosaicism and organismal lineages. *Trends Genet* **20**, 254–260.

Zverlov, V., Klein, M., Lücker, S., Friedrich, M. W., Kellermann, J., Stahl, D. A., Loy, A. & Wagner, M. (2005). Lateral gene transfer of dissimilatory (bi)sulfite reductase revisited. *J Bacteriol* **187**, 2203–2208.

Edited by: G. Muyzer