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Sulfur metabolisms in epsilon- and gamma-*Proteobacteria* in deep-sea hydrothermal fields

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In deep-sea hydrothermal systems, super hot and reduced vent fluids from the subseafloor blend with cold and oxidized seawater. Very unique and dense ecosystems are formed within these environments. Many molecular ecological studies showed that chemoautotrophic epsilon- and gamma-*Proteobacteria* are predominant primary producers in both free-living and symbiotic microbial communities in global deep-sea hydrothermal fields. Inorganic sulfur compounds are important substrates for the energy conservative metabolic pathways in these microorganisms. Recent genomic and metagenomic analyses and biochemical studies have contributed to the understanding of potential sulfur metabolic pathways for these chemoautotrophs. Epsilon-*Proteobacteria* use sulfur compounds for both electron-donors and -acceptors. On the other hand, gamma-*Proteobacteria* utilize two different sulfur-oxidizing pathways. It is hypothesized that differences between the metabolic pathways used by these two predominant proteobacterial phyla are associated with different ecophysiological strategies; extending the energetically feasible habitats with versatile energy metabolisms in the epsilon-*Proteobacteria* and optimizing energy production rate and yield for relatively narrow habitable zones in the gamma-*Proteobacteria*.

Keywords: deep-sea hydrothermal vents, energy metabolism, chemoautotroph

INTRODUCTION

The deep-sea hydrothermal system is one of the most extreme environments on Earth. It is characterized by darkness, high pressures, and steep physical and chemical gradients formed in mixing zones between hot hydrothermal vent fluid and cold deep-sea water. Very unique and dense communities of invertebrates are sustained in absence of a photosynthetic energy source (Dubilier et al., 2008). Almost all vent-endemic animals are strongly associated with the primary production of the endo- and/or epi-symbiotic chemoautotrophic microorganisms (Jeanthon, 2000). With the discovery of deep-sea hydrothermal ecosystem in 1977, it had been proposed that hydrogen sulfide-oxidizing and oxygen-reducing chemoautotrophs potentially sustain the primary production in these unique ecosystems (Kvenvolden et al., 1979). However, anoxic hydrothermal fluids contain several reduced compounds such as H_2, CH_4, and reduced metal ions in addition to H_2S (Jannasch and Mottl, 1985). Recent cultivation studies have demonstrated that these chemicals are all used as energy sources for chemoautotrophs (Jannasch and Mottl, 1985; Nakagawa et al., 2005; Campbell et al., 2006; Emerson et al., 2007), indicating the great diversity of chemoautotrophic energy metabolic processes in the ecosystems. Hydrogen gas (H_2) is one of the most important energy sources, and the hydrogen-dependent ecosystems may represent analogs for the earliest biological communities on Earth (Takai et al., 2004, 2006b; Nealson et al., 2005).

Hydrogen sulfide or sulfide is primarily supplied via high temperatures of seawater–rock interactions in the subseafloor hydrothermal reaction zones (Jannasch and Mottl, 1985). Thermodynamic modeling indicated that the hydrogen sulfide or sulfide abundantly contained in the hydrothermal fluids represented the dominating energy source in the mesophilic deep-sea vent chemoautotrophic ecosystems (McCollom and Shock, 1997). In addition, partially oxidized inorganic sulfur compounds such as polysulfide, elemental sulfur, and thiosulfate are generated in the *in situ* mixing zones, and serve as both electron donor and acceptor in a variety of energy metabolisms. The chemical and microbial oxidation and reduction reactions of sulfur compounds probably establish the overall complex sulfur metabolism network in the ecosystem. This article reviews the representative microbial components capable of utilizing the inorganic sulfur compounds as the energy sources in the deep-sea hydrothermal environments and highlights the biochemical and genetic components of their metabolisms. In addition, the possible segregation of different sulfur metabolic pathways and their host chemoautotrophic *Proteobacteria* associated with the physical and chemical transition in the mixing zones of the deep-sea hydrothermal environments is discussed.

MICROBIAL COMMUNITIES IN DEEP-SEA HYDROTHERMAL FIELDS

Recent culture-independent analyses of the both symbiotic and free-living microbial communities in the various deep-sea hydrothermal environments have revealed a great phylogenetic diversity of *Archaea* and *Bacteria* (Takai et al., 2006a); these analyses have also indicated a predominance in biomass of members within the gamma-*Proteobacteria* and epsilon-*Proteobacteria* (Stewart et al., 2005; Suzuki et al., 2005; Urakawa et al., 2005; Campbell et al., 2006; Nakagawa and Takai, 2008; **Table 1**). Based

Table 1 | Sulfur metabolic genes in epsilon- and gamma-*Proteobacteria* isolated or identified from deep-sea hydrothermal fields.

Organism, isolation, or observation site	Genome accession number*[1]	Growth temperature (°C)	Electron donor	Electron acceptor	Genes	Locus tag	Pathways	
EPSILON-*PROTEOBACTERIA*								
Sulfurovum sp. NBC37-1 Sediment, Mid-Okinawa Trough	AP009179	30–37	H_2, S^0, $S_2O_3^{2-}$	S^0, NO_3^-, O_2	*psrACB*	SUN0510-0508	Sulfur respiration	Nakagawa et al. (2005, 2007)
					soxXYZAB	SUN0497-0501	Sox system	Yamamoto et al. (2010)
					soxCDYZ	SUN0049-0052	Sox system	
					soxJ	SUN0058, 1338	Sox system (?)	
					sqr	SUN0047, 0073, 0192	Sulfide oxidation	
					sorAB	SUN1104-1103, 1476-1477	Sulfite oxidation	
Nitratiruptor sp. SB155-2 Sediment, Mid-Okinawa Trough	AP009178	55	H_2, S^0, $S_2O_3^{2-}$	S^0, NO_3^-, O_2	*psrACB*	NIS0573-0571	Sulfur respiration	Nakagawa et al. (2005, 2007)
					soxXYZAB	NIS1832-1828	Sox system	
					soxYZ	NIS0034-0035	Sox system	
					soxJ	NIS0032	Sox system (?)	
					sqr	NIS0146, 0158, 0326	Sulfide oxidation	
Nautilia profundicola AmH *Alvinella* tube, East Pacific Rise	CP001279	45	H_2, formate	S^0	*psrABC*	NAMH1518-1520	Sulfur respiration	Campbell et al. (2001, 2009)
Sulfurimonas autotrophica DSM16294 Sediment, Mid-Okinawa Trough	CP002205	25	H_2S, S^0, $S_2O_3^{2-}$	O_2	*psrACB*	Saut1622-1644	Sulfur respiration	Inagaki et al. (2003)
					soxXYZAB	Saut0991-0995	Sox system	
					soxCDYZ	Saut2096-2099	Sox system	
					soxJ	Saut1356	Sox system (?)	
					sqr	Saut0503, 1543	Sulfide oxidation	
					sorAB	Saut1022-1023	Sulfite oxidation	
Sulfurimonas denitrificans DSM1251 Tidal flat mud, Dutch Wadden Sea*[2]	CP000153	25	$S_2O_3^{2-}$	NO_3^-	*psrACB*	Suden0500-0498	Sulfur respiration	Sievert et al. (2008)
					soxXYZAB	Suden0260-0264	Sox system	
					soxCDYZ	Suden2060-2057	Sox system	
					soxJ	Suden2049	Sox system (?)	
					sqr	Suden0619	Sulfide oxidation	

Alvinella episymbiont East Pacific Rise	AAUQ	10-65	ND	ND	*psrAB*	ND	Sulfur respiration	Grzymski et al. (2008)
	01000000*3				*soxXYZABCD*	ND	Sox system	
					D	ND	Sox system (?)	
					soxJ			
GAMMA-*PROTEOBACTERIA*								
Thiomicrospira crunogena XCL-2 Galapagos Rift vent	CP000109	33	H_2, H_2S, S^0, $S_2O_3^{2-}$	O_2	*soxXYZA*	Tcr0604-0601	Sox system	Scott et al. (2006)
					soxB	Tcr1549	Sox system	
					soxCD	Tcr0156-0157	Sox system	
					sqr	Tcr0619	Sulfide oxidation	
CoSym *Vesicomyosocious* symbiont	AP009247	ND	ND	ND	*soxXYZA*	COSY0733-0730	Sox system	Kuwahara et al. (2007)
					soxB	COSY 0161	Sox system	
					soxJ	COSY0750	Sox system (?)	
					sqr	COSY0953	Sulfide oxidation	
					dsrAB	COSY0795-0794	Reverse sulfate reduction	
					aprAB	COSY0092-0091	Reverse sulfate reduction	
					sat	COSY0089	Reverse sulfate reduction	
Ruthia magnifica Calyptogena symbiont	CP000488	ND	ND	ND	*soxXYZA*	Rmag0808-0805	Sox system	Newton et al. (2007)
					soxB	Rmag0156	Sox system	
					soxJ	Rmag0824	Sox system (?)	
					sqr	Rmag1053	Sulfide oxidation	
					dsrAB	Rmag0870-0869	Reverse sulfate reduction	
					aprAB	Rmag0088-0087	Reverse sulfate reduction	
					sat	Rmag0085	Reverse sulfate reduction	
Endoriftia persephone Riftia symbiont	AASF	ND	ND	ND	*soxXAB**4	ND	Sox system	Robidart et al. (2008)
	00000001*3				*dsrAB*	ND	Reverse sulfate reduction	
					aprAB	ND	Reverse sulfate reduction	
					sat	ND	Reverse sulfate reduction	

(Continued)

Table 1 | Continued

Organism, isolation, or observation site	Genome accession number*[1]	Growth temperature (°C)	Electron donor	Electron acceptor	Genes	Locus tag	Pathways	
Beggiatoa spp. Sediment, Baltic Sea	ABBY	ND	ND	ND	*soxXYZAB*	ND	Sox system	Mußmann et al. (2007)
	00000000, ABBBZ 00000000[*5]				*fccAB*	ND	Sulfide oxidation	
					sqr	ND	Sulfide oxidation	
					dsrAB	ND	Reverse sulfate reduction	
					aprAB	ND	Reverse sulfate reduction	
					sat	ND	Reverse sulfate reduction	
					dmsABC	ND	DMSO respiration	
					phsABC	ND	Thiosulfate respiration	

ND no data, psr, polysulfide reductase; sox, Sox multi enzyme system; sqr, sulfide:quinone oxidoreductase; fcc, flavocytochrome c; dsr, dissimilatory sulfite reductase; apr, adenosine 5′-phosphosulfate reductase; sat, sulfate adenylyltransferase; dms, dimethyl sulfoxide reductase; phs, thiosulfate reductase.

*[*1] Deposited in DDBJ/EMBL/GenBank databases.*

*[*2] It is not a hydrothermal field.*

*[*3] For which the metagenome sequence is published.*

*[*4] SoxXAB were detected, but not SoxYZ.*

*[*5] Sequence assembles were incomplete.*

on the culture-dependent characterization and genetic components analyses, these *Proteobacteria* are found to be chemoautotrophs strongly associated with utilization of inorganic sulfur compounds (Durand et al., 1993; Inagaki et al., 2003, 2004; Takai et al., 2003b, 2006a,c). Therefore, the sulfur-related energy metabolisms in these *Proteobacteria* are important traits for understanding ecology and biogeochemistry in deep-sea hydrothermal vent ecosystems.

MICROBIAL SULFUR METABOLIC PATHWAYS FOR ENERGY CONSERVATION

Inorganic sulfur compounds are used as both electron donor and acceptor by microorganisms. Sulfur-oxidation pathways have been well studied biochemically in anaerobic phototrophs (e.g., *Chlorobium* and *Allochromatium*), facultatively chemoautotrophic *Proteobacteria* (e.g., *Acidithiobacillus* and *Paracoccus*) and *Sulfolobales* (e.g., *Sulfolobus* and *Acidianus*; Friedrich, 1998; Kletzin et al., 2004; Friedrich et al., 2005; Frigaard and Dahl, 2009). Bacterial sulfur-oxidation pathways under the neutral pH are generally classified into two different types: (1) reverse sulfate reduction, and (2) Sox multienzyme system. Reverse sulfate reduction is reversal of sulfate reduction pathway, in which sulfide is oxidized to sulfate via sulfite. This pathway uses the same families of enzymes as of the sulfate reduction pathway, such as dissimilatory sulfite reductase (Dsr), adenylphosphosulfate reductase (Apr), and sulfate adenylyltransferase (Sat; Kappler and Dahl, 2001). Sox multienzyme complex catalyzes oxidation of inorganic sulfur compounds. Four protein components, SoxYZ, SoxXA, SoxB, and SoxCD are required for complete oxidation of sulfide and thiosulfate to sulfate (Complete Sox pathway; Friedrich et al., 2001). However, the sulfur oxidizers that possess the *soxYZXAB* genes but not *soxCD* have often been reported (Friedrich et al., 2005). It is considered that SoxCD acts as sulfur dehydrogenase. Sox system without SoxCD is able to oxidize sulfite and sulfone group (- SO_3^-) in thiosulfate, but not sulfide, elemental sulfur, and sulfane sulfur (-S^-) in thiosulfate (Friedrich et al., 2001). In addition to these two sulfur-oxidation pathways, several enzymes have been proposed to oxidize inorganic sulfur compounds, including sulfide:quinone oxidoreductase (Sqr), flavocytochrome *c* (Fcc), and sulfite oxidase (SO), though the exact physiological functions are still debated. Sqr is a single-subunit flavoprotein that catalyzes oxidation of sulfide to elemental sulfur (Shahak and Hauska, 2008; Frigaard and Dahl, 2009). Recent protein structural study divided Sqr family into six groups (Marcia et al., 2010). These different types of Sqr probably have different mechanisms and physiological roles (Chan et al., 2009; Gregersen et al., 2011; Holkenbrink et al., 2011). Fcc also catalyzes oxidation of sulfide to elemental sulfur, but electrons are transferred to the level of cytochrome *c* (Oh-oka and Blankenship, 2004). Fcc consists of a flavoprotein subunit, FccB, and a c-type cytochrome subunit, FccA (Kusai and Yamanaka, 1973; Reinartz et al., 1998). The *soxEF* genes conserved in the *sox* gene clusters are homologous genes of the *fccAB* genes (Friedrich et al., 2008). The *soxJ* gene is a homologous gene of *fccB/soxF*, that does not cluster with a homolog of *fccA/soxE* (Gregersen et al., 2011). SO is a molybdenum enzyme and catalyzes the direct oxidation of sulfite to sulfate (Kappler and Dahl, 2001). Acidophilic sulfur-oxidizing bacteria and archaea use different sulfur-oxidation pathways that will not be further described here (see Ghosh and Dam, 2009).

Sulfur-reduction pathways can be classified into two types based on the substrates: (1) sulfur-reduction (sulfur respiration), and (2) sulfate reduction (sulfate respiration). Sulfur-reduction pathways have been intensively studied in gastrointestinal epsilon-*Proteobacterium*, *Wolinella succinogenes* (Wolin et al., 1961; Hedderich et al., 1999). Sulfur-reduction is catalyzed by polysulfide reductase (Psr), in which the actual substrate of the reaction is polysulfide but not elemental sulfur (Pfennig and Biebel, 1986). Sulfate reducing bacteria are found in several different phylogenetic lines (e.g., delta-*Proteobacteria*, *Clostridia*, *Thermodesulfobacteria*, and *Nitrospirae*; Muyzer and Stams, 2008). Sat, Apr, and Dsr catalyze the reduction of sulfate to sulfide.

SULFUR METABOLISM IN EPSILON-*PROTEOBACTERIA* IN DEEP-SEA HYDROTHERMAL FIELDS

The most well known epsilon-*Proteobacteria* are gastrointestinal pathogens such as *Helicobacter* and *Campylobacter*. They are heterotrophic and do not have inorganic sulfur metabolic pathways. The genus *Wolinella* isolated from cattle rumen can reduce elemental sulfur to hydrogen sulfide (Wolin et al., 1961).

There are many reports that chemoautotrophic epsilon-*Proteobacteria* are predominant in sulfidic environments such as deep-sea hydrothermal and subsurface environments (Takai et al., 2004; Campbell et al., 2006; Nakagawa and Takai, 2008). Recently, whole genome sequences of five strains of marine epsilon-*Proteobacteria*, i.e., *Sulfurovum* sp. NBC37-1, *Nitratiruptor* sp. SB155-2, *Nautilia profundicola* AmH, *Sulfurimonas autotrophica* DSM16294 isolated from deep-sea hydrothermal fields, and *S. denitrificans* DSM1251 isolated from a coastal wetland, have been determined (Nakagawa et al., 2007; Sievert et al., 2008; Campbell et al., 2009; **Table 1**). Moreover, the metabolism of episymbiontic epsilon-*Proteobacterium* in a polychaete worm was analyzed by metagenomic analysis (Grzymski et al., 2008). In addition, several biochemical studies on sulfur metabolic pathways in deep-sea epsilon-*Proteobacteria* have also been reported (Takai et al., 2005; Yamamoto et al., 2010).

Genetic and enzymatic components characterized by the genomic and biochemical analyses of deep-sea epsilon-*Proteobacteria* have facilitated considerable progress in determining the possible sulfur metabolism pathways of the deep-sea chemoautotrophic epsilon-*Proteobacteria* (**Table 1**). The deep-sea epsilon-*Proteobacteria* characterized so far possess (1) a hydrogen-oxidizing sulfur respiration pathway using hydrogenase and polysulfide reductase (Psr). And the epsilon-*Proteobacteria* except for *N. profundicola* AmH, possess (2) a sulfur compounds-oxidizing oxygen/nitrate-respiration pathway using the Sox multienzyme system. Both of the Sox systems coupled with and without SoxCD have been found. The *sox* genes are not organized in a single region, but at least in two separated regions of the *soxXYZAB* and *sox*(*CD*)*YX* genes. One or more *soxF* gene was conserved in each sox gene cluster. *Sulfurovum* sp., *Nitratiruptor* sp., and *S. autotrophica* have several *sqr* genes. Two sets and one set of the *sorAB* genes coding SO were observed in *Sulfurovum* sp. and *S. autotrophica*, respectively. No gene for sulfur-oxidation pathways was found in *N. profundicola*. In addition to the sulfur metabolic

pathways, many deep-sea epsilon-*Proteobacteria* are capable of chemoautotrophic growth without sulfur compounds using a third metabolic pathway: (3) a hydrogen-oxidizing oxygen/nitrate-reduction pathway (**Figure 1**). The bacterial members that can use sulfur compounds as both electron acceptors and donors are identified only in the phyla of epsilon-*Proteobacteria* and *Aquificae*, both of which predominantly inhabit in deep-sea hydrothermal environments. This may provide an important clue into how the versatile energy metabolic pathways are associated with the energetic advantages adapted to the dynamic and transient environmental conditions in the mixing zones of the hydrothermal vent environments.

SULFUR METABOLISM IN GAMMA-*PROTEOBACTERIA* IN DEEP-SEA HYDROTHERMAL FIELDS

Gamma-proteobacterial symbionts with invertebrates have never been isolated from deep-sea hydrothermal vents. Furthermore only a couple of free-living sulfur-oxidizing gamma-*Proteobacteria* have been isolated from deep-sea hydrothermal fields (Kuever et al., 2002; Takai et al., 2006c, 2009). Unculturable gamma-proteobacterial sulfur-oxidizing endosymbionts are classified into three groups based on their host invertebrates: symbionts of (1) *Bathymodiolus* mussels and *Calyptogena* clams, (2) *Alvinicon-cha* gastropods, and (3) gastropods (e.g., *Ifremeria*) and tube-worms (Nakagawa and Takai, 2008). In recent years, two genome sequences of sulfur-oxidizing symbionts in *Calyptogena* have been published (Kuwahara et al., 2007; Newton et al., 2007; **Table 1**). Moreover, the metabolisms of symbiont in tubeworm were analyzed by metagenomic analysis (Robidart et al., 2008). In addition, a whole genome sequence of free-living sulfur-oxidizing gamma-*Proteobacterium*, *Thiomicrospira crunogena* XCL-2 was also published (Scott et al., 2006). Putative metabolic pathways in *Beggiatoa* sp. from marine sediments had also analyzed by using incomplete genome sequence (Mußmann et al., 2007). From these genome-based genetic characteristics, we can develop a preliminary outline of representative sulfur-related energy metabolisms in the deep-sea hydrothermal gamma-*Proteobacteria*, which possess two different sulfur-oxidization pathways: (1) the reverse sulfate reduction using Dsr, Apr, and Sat, and (2) the Sox multienzyme system without SoxCD (**Figure 1**). *T. crunogena* XCL-2, however, is an exception, in which no gene for the reverse sulfate reduction pathway was found. Moreover, this organism has the *soxCD* genes separated from other *sox* genes. In contrast, functional genes amplification studies suggested that hydrothermal gamma-*Proteobacteria* use the reverse sulfate reduction pathway, but not the Sox pathway (Hügler et al., 2010). These results indicate that either of the two distinct sulfur-oxidization pathways is dispensable. Both of these pathways require O_2 as a terminal electron acceptor in most cases. This fact indicates that the relatively reductive environment (O_2-depleted condition) is inhibitory for the growth of deep-sea chemoautotrophic gamma-*Proteobacteria* due to the limitation of primary electron acceptor despite an increasing abundance of electron-donors (reduced sulfur compounds). Thus, it is predicted that the metabolically habitable spaces of deep-sea chemoautotrophic gamma-*Proteobacteria* strictly requiring co-existence of reduced sulfur compounds and O_2 are much more limited than those of deep-sea epsilon-*Proteobacteria* in the mixing zones of hydrothermal environments. Conversely, however, it seems likely that the genomic installation and the biochemical operation of two different sulfur-oxidizing pathways in the deep-sea chemoautotrophic gamma-*Proteobacteria* are kinetically advantageous if both the reduced sulfur compounds and O_2 are steadily supplied into the habitats. Particularly for the gamma-proteobacterial sulfur-oxidizing symbionts, the host invertebrates would prepare the metabolically suitable habitats in and around the bodies in the mixing zones of deep-sea hydrothermal environments (Arp and Childress, 1983; Zal et al., 1998; Numoto et al., 2005). This may be one of the possible rationales for the domination of deep-sea gamma-proteobacterial sulfur-oxidizing chemoautotrophs in symbiotic communities in deep-sea hydrothermal environments. All deep-sea sulfur-oxidizing gamma-*Proteobacteria* possess one *sqr* gene in the genome, except for the metagenome of *Riftia* symbiont. Therefore, Sqr probably has an important role for the sulfur-oxidation in the organisms. It is interesting that genes for sulfur compound (thiosulfate and dimethyl sulfoxide) respiration were found in genome of *Beggiatoa* sp. (Mußmann et al., 2007). This is in accordance with previous results in *Beggiatoa alba* that can reduce stored elemental sulfur to overcome short-term anoxic conditions (Nelson and Castenholz, 1981; Schmidt et al., 1987).

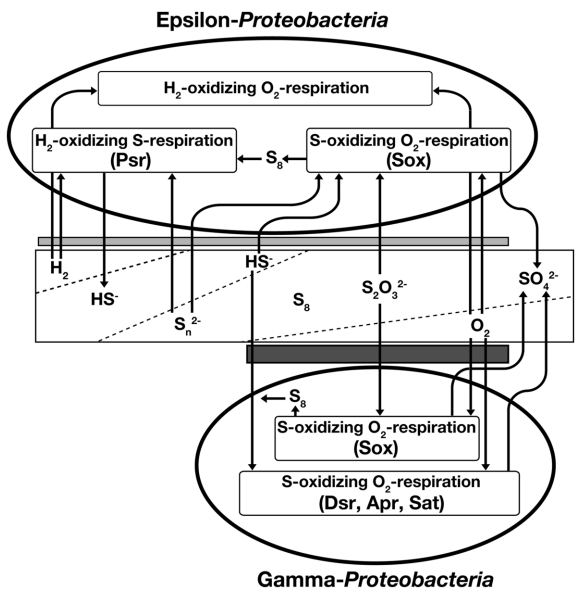

FIGURE 1 | Energy metabolic pathways and habitat area of epsilon-*Proteobacteria* and gamma-*Proteobacteria* in deep-sea hydrothermal fields. From left to right on the diagram, the redox conditions shift from reductive to oxidative states. Broken lines in the rectangle indicate inclines in concentration of hydrogen gas, hydrogen sulfide, oxidized sulfur compounds, and oxygen gas, respectively. Two gray bars indicate habitat area of epsilon-*Proteobacteria* and gamma-*Proteobacteria*, respectively. Psr, polysulfide reductase; Sox, Sox multienzyme system; Dsr, dissimilatory sulfite reductase; Apr, adenosine 5′-phosphosulfate reductase; Sat, sulfate adenylyltransferase.

CONCLUDING REMARKS

In this review, we described the sulfur-related energy metabolisms of epsilon- and gamma-*Proteobacteria* dominating the microbial

communities in the global deep-sea hydrothermal environments. Most of the metabolic characteristics summarized in this article are based on the reconstruction of genetic components in the genomes determined for only a few representative species. Further genetic and biochemical variability associated with the sulfur-related energy metabolisms will be clarified in many species of deep-sea hydrothermal vent epsilon- and gamma-*Proteobacteria* in the future. Surely, the investigation of other inorganic sulfur metabolizing groups of microbial components such as sulfate-reducers (delta-*Proteobacteria, Thermodesulfobacteria,* and *Deferribacteres*) and sulfur-reducing thermophiles (*Aquificae, Thermococcaceae,* and *Desulfurococcaceae*) will be important for understanding the biogeochemical processes and energy- and elemental fluxes of sulfur compounds in the deep-sea hydrothermal environments (Reysenbach et al., 2000, 2002; Teske et al., 2002; Takai et al., 2003a). Recently, genetic information has been rapidly accumulating and has been providing the important genetic potential for understanding the complex sulfur-related metabolic networks in deep-sea hydrothermal vent ecosystems. In contrast, enzymatic and biochemical data are highly insufficient to clarify the *in vivo* and *in situ* kinetics, operation and control of metabolic pathways. These will be an important focus for future studies.

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