MINIREVIEW

Deep-sea vent chemoautotrophs: diversity, biochemistry and ecological significance

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Received 26 January 2008; revised 4 April 2008; accepted 10 April 2008.
First published online 22 May 2008.

DOI:10.1111/j.1574-6941.2008.00502.x

Editor: Gary King

Keywords
chemoautotroph; deep-sea vents; carbon fixation; energy metabolism.

Abstract

Deep-sea vents support productive ecosystems driven primarily by chemoautotrophs. Chemoautotrophs are organisms that are able to fix inorganic carbon using a chemical energy obtained through the oxidation of reduced compounds. Following the discovery of deep-sea vent ecosystems in 1977, there has been an increasing knowledge that deep-sea vent chemoautotrophs display remarkable physiological and phylogenetic diversity. Cultivation-dependent and -independent studies have led to an emerging view that the majority of deep-sea vent chemoautotrophs have the ability to derive energy from a variety of redox couples other than the conventional sulfur–oxygen couple, and fix inorganic carbon via the reductive tricarboxylic acid cycle. In addition, recent genomic, metagenomic and postgenomic studies have considerably accelerated the comprehensive understanding of molecular mechanisms of deep-sea vent chemoautotrophy, even in yet uncultivable endosymbionts of vent fauna. Genomic analysis also suggested that there are previously unrecognized evolutionary links between deep-sea vent chemoautotrophs and important human/animal pathogens. This review summarizes chemoautotrophy in deep-sea vents, highlighting recent biochemical and genomic discoveries.

Introduction

Most microbial species require organic compounds for their carbon and energy sources and are referred to as heterotrophs. Autotrophs, by contrast, are able to assimilate inorganic carbon as their carbon source. The energy required for carbon assimilation is derived either from sunlight (photoautotrophy) or from the oxidation of inorganic-reduced compounds (chemoautotrophy). Deep-sea vents are representative areas on the seafloor of high biological productivity fuelled primarily by microbial chemoautotrophy. Energy and carbon sources, e.g., H_2, H_2S, CO and CO_2, are supplied by magma degassing and/or from reactions between seawater and rock at high temperatures (Reysenbach & Shock, 2002). In the present review, we will not discuss carbon monoxide as an energy source because relatively little is known about carboxydotrophy in deep-sea vents (Sokolova *et al.*, 2001).

Deep-sea vent chemoautotrophs are of particular interest as chemoautotrophy-based ecosystems may represent analogs for the earliest biological communities on Earth or for possible extraterrestrial life (Nealson *et al.*, 2005). Given the variety in physical and chemical conditions of deep-sea vents, it is not surprising that chemoautotrophs inhabiting these environments exhibit considerable physiological diversity. However, until recently, cultured deep-sea vent chemoautotrophs comprised mostly thermophiles and hyperthermophiles, for which substantial habitats are restricted to proximate, high-temperature vent fluids, e.g. chimney structures (Nakagawa & Takai, 2006).

Studies on mesophilic deep-sea vent chemoautotrophs have focused on gammaproteobacterial endosymbionts of various vent fauna, for which pure cultures are still unavailable (Van Dover, 2000). In a recent breakthrough, pure cultures of mesophilic to moderately thermophilic deep-sea vent *Epsilonproteobacteria*, a dominant bacterial group in endosymbiotic to episymbiotic associations with various vent fauna, demonstrated that the majority were versatile chemoautotrophs capable of oxidation of H_2 and sulfur compounds coupled with the reduction of oxygen, nitrate

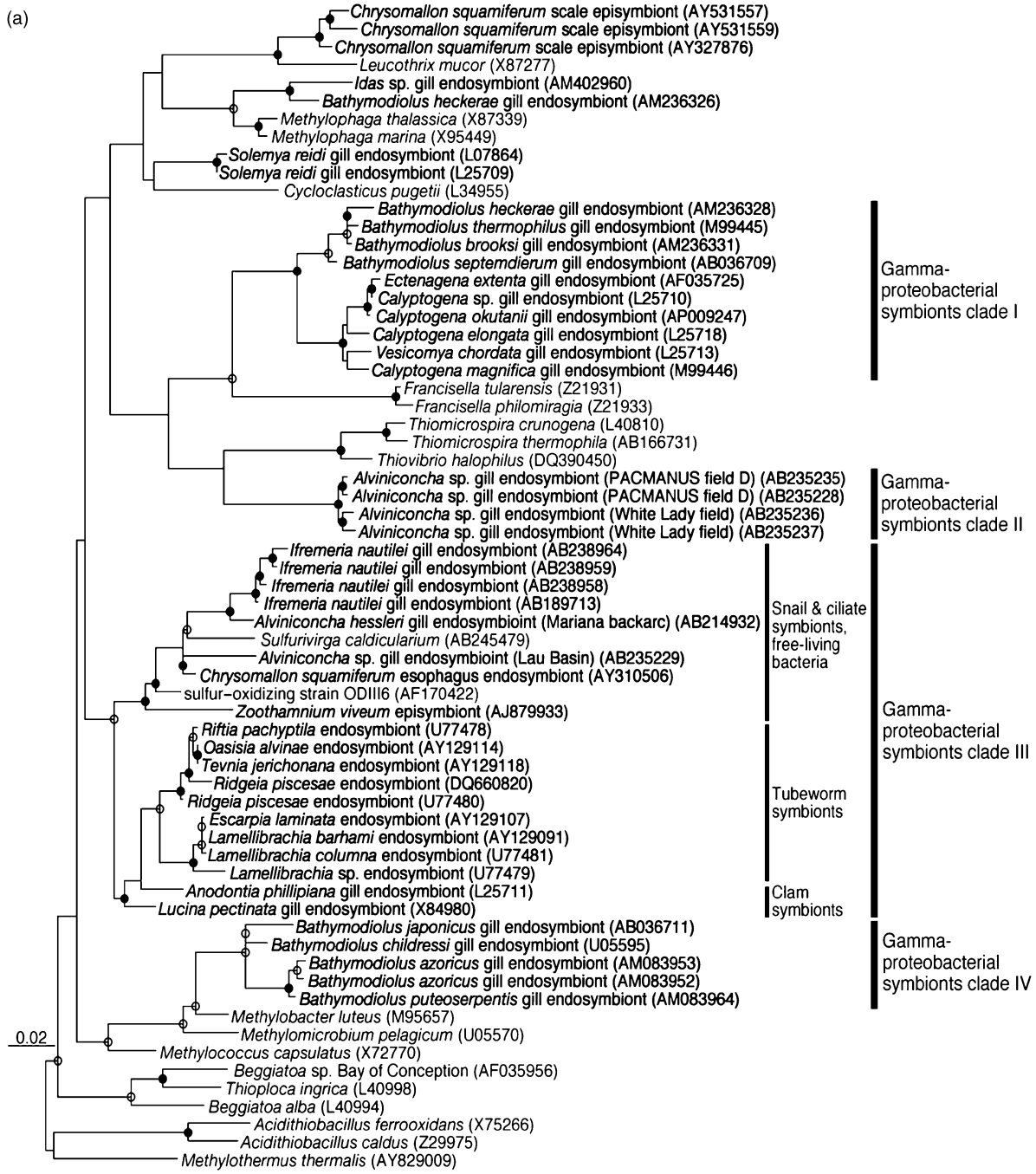

Fig. 1. 16S rRNA gene-based trees showing the major mesophilic deep-sea vent chemoautotrophs. The trees were constructed, evaluated and optimized using the ARB program (Ludwig *et al*., 2004). Chemoautotrophic symbionts are indicated in bold type. GenBank/DDBJ/EMBL accession numbers are shown in parentheses. The scale bars represent the expected number of changes per nucleotide position. Distances were estimated with the Jukes–Cantor correction. Bootstrap analyses with 100 trial replications were used to obtain confidence estimates for the tree topologies. Branch points conserved with bootstrap values of $> 75\%$ (•) and with bootstrap values of $> 50\%$ (○) are indicated. (a) Tree showing the relationships among members of the *Gammaproteobacteria*. Unambiguously aligned positions (945 bases) were used. (b) Tree showing the relationships among members of the *Epsilonproteobacteria*. Unambiguously aligned positions (1026 bases) were used.

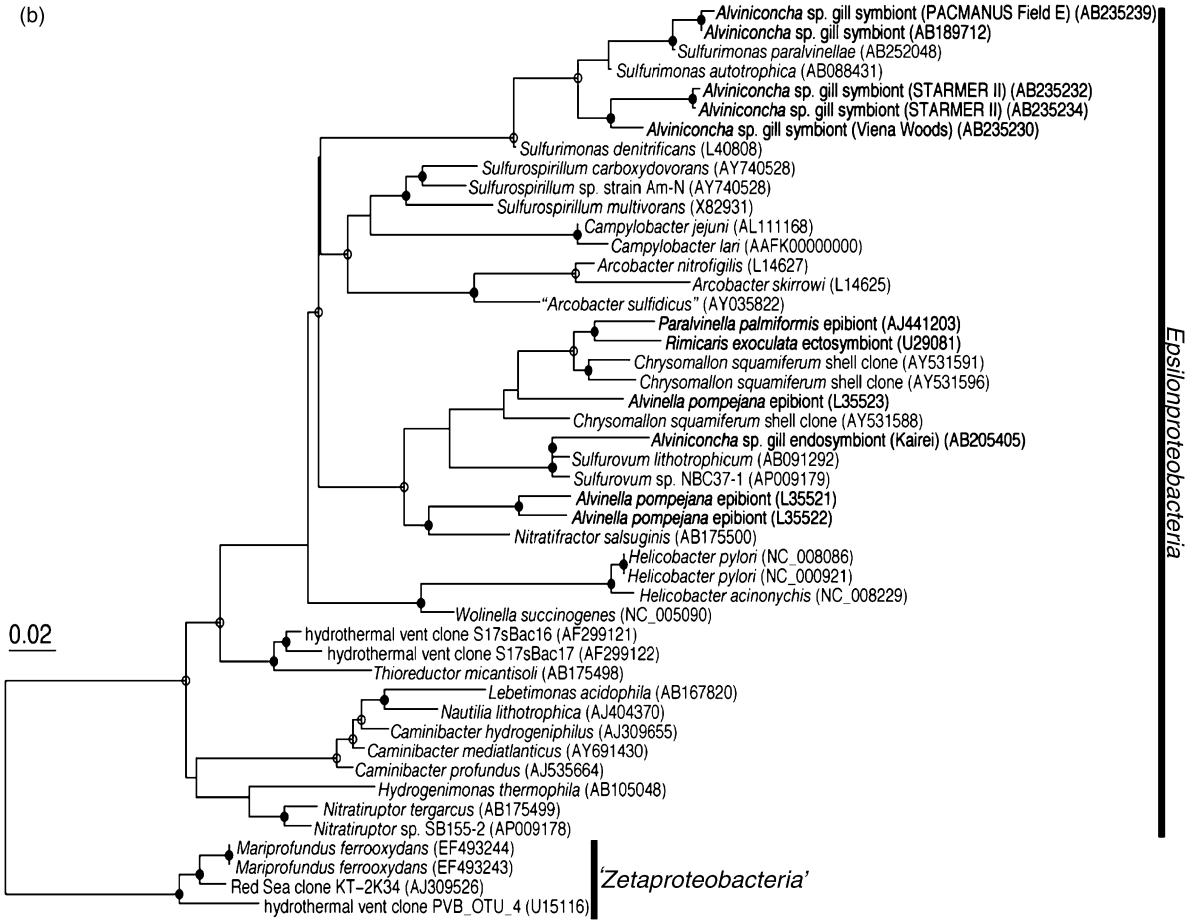

Fig. 1. (Continued)

and sulfur compounds (Campbell *et al.*, 2001; Takai *et al.*, 2003; Nakagawa *et al.*, 2005b, c). In addition, recent successful isolation of a mesophilic, ammonia-oxidizing marine group 1 crenarchaeon (Könneke *et al.*, 2005), a dominant archaeal group in the global ocean, including seawater surrounding deep-sea vents (Takai *et al.*, 2004b), suggested that archaeal chemoautotrophy is of ecological and biogeochemical significance not only at the local scale but also globally in the deep-sea water column (Wuchter *et al.*, 2006). More recently, a novel chemoautotrophic bacterium, *Mariprofundus ferooxydans*, was isolated and characterized from metalliferous deposits at Loihi Seamount (Emerson *et al.*, 2007). The new isolate is a strictly aerobic Fe(II) oxidizer and represents a new class of *Proteobacteria* ('*Zetaproteobacteria*'). In addition to its unique phylogenetic position, its biogeochemical significance is also striking. As Fe(II) is abundantly present in the young (<65 Ma) oceanic basaltic crust, this type of chemoautotrophy may play an important role in the global cycling of carbon and iron (Bach & Edwards, 2003). In addition, recent genomic, metagenomic and postgenomic studies of isolated or environmental chemoautotrophs have rapidly and comprehensively revealed the molecular mechanisms associated with deep-sea vent chemoautotrophy (Scott *et al.*, 2006; Kuwahara *et al.*, 2007; Markert *et al.*, 2007; Nakagawa *et al.*, 2007; Newton *et al.*, 2007). The present review summarizes chemoautotrophy in deep-sea vents, highlighting recent findings in genomics and biochemistry.

Phylogeny and distribution of mesophilic deep-sea vent chemoautotrophs

As the phylogeny and distribution of thermophilic and hyperthermophilic deep-sea vent chemoautotrophs have been described elsewhere (Nakagawa & Takai, 2006), we focus here on mesophiles, largely comprising symbionts of vent fauna. In general, chemoautotrophic endosymbionts and episymbionts belong to the *Gammaproteobacteria* (Fig. 1a) and *Epsilonproteobacteria* (Fig. 1b), respectively. Although close relatives of epsilonproteobacterial symbionts (both episymbionts and endosymbionts) are now culturable, gammaproteobacterial chemoautotrophs isolated from

deep-sea vents are only distantly related to any chemoautotrophic symbionts (Fig. 1a). The unculturable gammaproteobacterial symbionts can be classified into four major clades: (1) clade I including sulfur-oxidizing endosymbionts of *Bathymodiolus* mussels and *Calyptogena* clams, (2) clade II including sulfur-oxidizing endosymbionts of *Alviniconcha* gastropods, (3) clade III including sulfur-oxidizing endosymbionts of gastropods and tubeworms, and (4) clade IV including methane-oxidizing endosymbionts of *Bathymodiolus* (Fig. 1a). It is notable that gammaproteobacterial symbiont clade III includes several free-living isolates from shallow-water hydrothermal vents (Fig. 1a; Kuever *et al.*, 2002; Takai *et al.*, 2006a).

The occurrence of vent fauna hosting chemoautotrophic symbionts (e.g. vestimentiferan tubeworms, bivalve mollusks, provannid gastropods, alvinellid polychaete and bresiliid shrimps) in various hydrothermal niches indicates that chemoautotrophic symbiosis is diverse and probably adapted to various deep-sea vent environments (Table 1). The intrafield distribution of specific taxa in different colonies could be controlled by physical and chemical factors including temperature, pH, redox potential and concentrations of oxygen, iron, nitrate, ammonium and sulfur compounds (Lee *et al.*, 1999; Luther *et al.*, 2001). The global and local biogeography of vent fauna have been well studied, characteristics of which probably result from various factors including geologic setting (e.g. tectonic history and oceanic circulation patterns) and geochemistry of vent fluids (Van Dover *et al.*, 2002). Among vent fauna, *Bathymodiolus* mussels and *Alviniconcha* gastropods are unique in that they have a wide range of endosymbionts (Fig. 1). In some cases (e.g. *Bathymodiolus azoricus* and *Bathymodiolus puteoserpentis* from the Mid-Atlantic Ridge), clade I and clade IV members coexist in the same host cell, indicating that current symbiotic associations result from repeated and independent symbiotic events (DeChaine & Cavanaugh, 2005). In contrast, *Calyptogena* clams are known to transmit their endosymbionts vertically (Hurtado *et al.*, 2003). Although *Calyptogena* endosymbionts still retain almost complete gene sets for carbon metabolism (Kuwahara *et al.*, 2007; Newton *et al.*, 2007), reductive evolution was discovered in the endosymbiont genomes (Kuwahara *et al.*, 2008) as in the reduced genomes of vertically transmitted endosymbionts of insects (Dale & Moran, 2006). Although the vertically transmitted *Calyptogena* endosymbionts and horizontally acquired *Bathymodiolus* endosymbionts are closely related (Fig. 1a), comparative genomic analysis would reveal significant differences in their genomes. The biogeography of free-living deep-sea vent chemoautotrophs is much less clear. However, the link between chemoautotrophic microbial community structures and geologic/geochemical settings of hydrothermal systems and their associated habitats has become increasingly evident (Takai *et al.*, 2004a, 2006b; Nakagawa *et al.*, 2005a, b; Huber *et al.*, 2007). In particular, the significance of certain geochemical constraints to energy metabolism (thermodynamic and/or kinetic aspects) has been suggested (McCollom & Shock, 1997; Takai *et al.*, 2006b).

Carbon fixation pathway

There are four major pathways for CO_2 fixation, i.e. the Calvin–Benson–Bassham (CBB) cycle, reductive tricarboxylic acid (rTCA) cycle (Arnon cycle), 3-hydroxypropionate (3-HP) cycle and reductive acetyl coenzyme A (acetyl-CoA) pathway (Wood–Ljungdahl pathway) (Fig. 2). In addition, a variant of the 3-HP cycle, designated 3-hydroxypropionate/4-hydroxybutyrate (3-HP/4-HB) cycle, was recently characterized in detail in a thermoacidophilic archaeon, *Metallosphaera sedula* (Berg *et al.*, 2007). Energy expenditures required to fix CO_2 differ among pathways (Fig. 2). The CBB cycle requires three molecules of ATP for the fixation of one CO_2, while the rTCA cycle requires one ATP for one CO_2. In the reductive acetyl-CoA pathway, used by chemoautotrophs inhabiting H_2-rich environments, CO_2 is directly reduced by H_2 and thus ATP input is not required. Chemoautotrophs may have evolved to adopt the most energetically parsimonious CO_2 fixation pathway available given the thermodynamic constraints of the cost of biomass synthesis (McCollom & Amend, 2005). Stable carbon isotopic compositions of biomass, and the presence or absence of key enzymes of each pathway have been used to diagnose the CO_2 fixation pathway, especially for unculturable endosymbionts (Van Dover & Fry, 1989; House *et al.*, 2003). In general, the CBB cycle and reductive acetyl-CoA pathway yield higher fractionations than other cycles (described below).

CBB cycle

The CBB cycle is probably the most prevalent pathway of CO_2 fixation on Earth and has been found in a variety of autotrophs, including most photoautotrophs (e.g. *Rhodobacter*, *Chromatium*, *Cyanobacteria* and all eukaryotic plants) and chemoautotrophs (e.g. *Thiobacillus* and *Hydrogenovibrio*) (Shively *et al.*, 1998). Unlike other CO_2 fixation pathways, the CBB cycle is used exclusively by *Bacteria* and *Eukaryota*. In this energetically expensive pathway, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is a key enzyme which catalyzes the carboxylation or oxygenation of ribulose-1,5-bisphosphate (RuBP) with CO_2 or O_2 (Fig. 2a). The RuBisCOs are classified into four groups, i.e. form I to IV. Form I RuBisCO, the most prevalent form in nature, is a hexadecamer of eight large and small subunits (L_8S_8). Form II RuBisCO, a dimer of large subunits (L_2), has a lower specificity for CO_2 and thus is more effective under CO_2-rich conditions (Tabita *et al.*, 2007). The RuBisCOs,

Table 1. Physiological and phylogenetic characteristics of major deep-sea vent chemoautotrophs

Phylogenetic group (representative genera)	Growth temperature range of isolates (°C)	CO_2 fixation pathway	Energy source	Major habitat	Host animal (type of symbiosis; bacteriocyte)	Relationship to oxygen
Bacteria						
Epsilonproteobacteria (*Sulfurimonas**, *Hydrogenimonas*, *Caminibacter**, *Lebetimonas*, *Nautilia**, *Nitratifractor*, *Nitratiruptor**, *Sulfurovum**, *Thioreductor*, *Arcobacter**)	4–70	rTCA	Hydrogen, sulfur compounds	Mixing zone	*Alviniconcha* gastropods (Endosymbiosis; gill) Alvinellid polychaete (Episymbiosis; dorsal integment) Bresilioid shrimps (Episymbiosis; branchial chamber, mouth) *Chrysomallon* gastropods (Episymbiosis; iron sulfide scale)	Strictly anaerobic to facultatively aerobic
Gammaproteobacteria (*Thiomicrospira**, *Halothiobacillus*, *Beggiatoa**, clade I members*, clade II members, clade III members*)	10–55	CBB	Sulfur compounds	Mixing zone	Vestimentiferan tubeworms (Endosymbiosis; trophosome) Bivalve mollusks (Endosymbiosis; gill) *Alviniconcha* and *Ifremeria* gastropods (Endosymbiosis; gill) *Chrysomallon* gastropods (Endoisymbiosis; esophagus)	Facultatively aerobic to strictly aerobic
Alphaproteobacteria (several unclassified strains)	3–30	ND	Ferrous iron	Metalliferous deposit	–	Facultatively aerobic
'*Zetaproteobacteria*' (*Mariprofundus**)	3–30	CBB	Ferrous iron	Metalliferous deposit	–	Strictly aerobic
Thermodesulfobacteria (*Thermodesulfobacterium**, *Thermodesulfatator**)	50–80	Unknown	Hydrogen	Chimney structure	–	Strictly anaerobic
Aquificae (*Persephonella**, *Aquifex**, *Balnearium*, *Desulfurobacterium**, *Thermovibrio**)	40–80	rTCA	Hydrogen, sulfur-compounds	Chimney structure	–†	Strictly anaerobic to facultatively aerobic
Deferribacteres (*Deferribacter**)	40-70	Unknown	Hydrogen, organic matter	Chimney structure	Bresilioid shrimps (Episymbiosis?; gut)	Strictly anaerobic
Archaea						
Marine group I *Crenarchaeota* (*Nitrosopumilus**, *Cenarchaeum**)	ND	3-HP/4-HB?	Ammonium	Deep-sea water column	–	Strictly aerobic
Thermoprotei (*Pyrodictium**, *Ignicoccus**, *Pyrolobus**)	70–113	rTCA variant?	Hydrogen	Chimney structure	–	Strictly anaerobic to facultatively aerobic
Archaeoglobales (*Archaeoglobus**, *Geoglobus*)	65–90	3-HP/4-HB?	Hydrogen, organic matter	Chimney structure	–	Strictly aerobic
Methanococci (*Methanococcus**, *Methanothermococcus**, *Methanotorris**, *Methanocaldococcus**)	17–92	reductive acety-CoA	Hydrogen	Chimney structure	–†	Strictly anaerobic
Methanopyri (*Methanopyrus**)	84–110	reductive acetyl-CoA	Hydrogen	Chimney structure	–	Strictly anaerobic

*For which the genome sequence is available or being sequenced.

†Abundantly found in tubes of alvinellid polychaetes Nakagawa *et al*. (2005b).

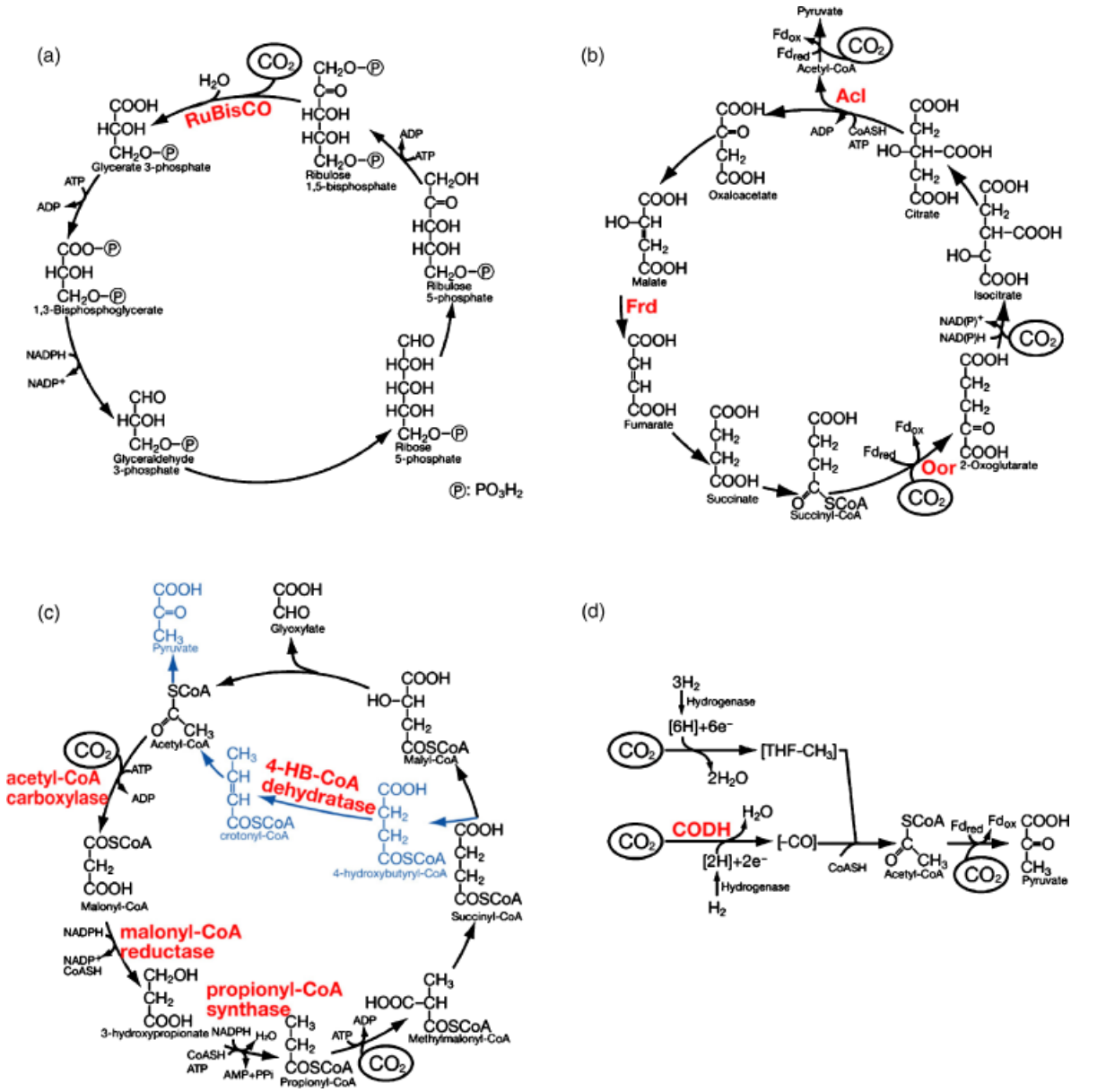

Fig. 2. Four major pathways of CO_2 fixation. Key enzymes are shown in red. (a) CBB cycle, (b) rTCA cycle, (c) 3-HP and 3-HP/4-HB (shown in blue) cycle, (d) reductive acetyl-CoA pathway.

both form I and II in most cases, have been identified enzymatically or genetically in various deep-sea vent chemoautotrophs (Table 1), including free-living, sulfur-oxidizing *Gammaproteobacteria* (e.g. *Thiomicrospira* and *Beggiatoa*; Scott *et al.*, 2006; Mussmann *et al.*, 2007), an iron-oxidizing 'zetaproteobacterium' (i.e. *Mariprofundus*; Emerson *et al.*, 2007), and gammaproteobacterial endosymbionts of tubeworms (e.g. *Riftia*; Felbeck, 1981), mussels (e.g. *Bathymodiolus*; Robinson *et al.*, 1998), gastropods (e.g. *Alviniconcha* from the Mariana backarc basin; Stein *et al.*, 1990) and clams (e.g. *Calyptogena*; Kuwahara *et al.*, 2007; Newton *et al.*, 2007) (Table 1). All of the CBB cycle-operating deep-sea vent chemoautotrophs are mesophiles inhabiting transition zones between reducing hydrothermal vents and oxic peripheral zones (Van Dover *et al.*, 2002). The carbon isotopic fractionations between CO_2 and biomass have been determined to be in the range 27–35‰ by form I RuBisCOs and 9–15‰ by form II RuBisCOs (Robinson *et al.*, 2003). Although relatively ^{13}C-enriched biomass of *Ridgeiidae* (e.g. *Riftia* and *Ridgeia*) and *Tevniidae* (e.g. *Tevnia* and *Oasisia*) tubeworms ($\delta^{13}C$ of -16‰ to -9‰) has long been debated (Nelson & Fisher, 1995), recent

metagenomic and proteomic analysis suggested that the *Riftia pachyptila* endosymbiont is the first chemoautotroph to have components of both CBB and rTCA cycles (Markert *et al*., 2007). Further biochemical studies are needed to clarify the CO_2 fixation pathway used by tubeworm endosymbionts.

Genome analysis suggested the presence of form III RuBisCO in deep-sea vent-inhabiting hyperthermophilic *Euryarchaeota* (e.g. *Methanocaldococcus*, *Archaeoglobus* and *Thermococcus*; Klenk *et al*., 1997; Fukui *et al*., 2005). The form III RuBisCO of *Thermococcus kodakaraensis* is involved in AMP metabolism (Sato *et al*., 2007). Similarly, the form IV RuBisCO, called RuBisCO-like protein (RLP), in an rTCA-utilizing phototroph, *Chlorobium tepidum*, is involved not in carbon fixation but in sulfur metabolism and its response to oxidative stress (Hanson & Tabita, 2001). It is suggested that all photosynthetic RuBisCOs and RLPs have evolved from a form III RuBisCO of a methanogenic archaeon (Tabita *et al*., 2007).

rTCA cycle

The rTCA cycle was originally discovered in green sulfur phototrophs (i.e. *Chlorobium*; Evans *et al*., 1966), and has been identified in diverse chemoautotrophs including a sulfate-reducing deltaproteobacterium (i.e. *Desulfobacter hydrogenophilus*), thermophilic *Aquificales* (e.g. *Hydrogenobacter* and *Aquifex*) and *Thermoproteales* (e.g. *Thermoproteus*) (Hügler *et al*., 2007). The rTCA cycle is essentially a reversal of the oxidative TCA or Krebs cycle, by which most aerobic heterotrophs oxidize organic matter. The rTCA cycle-specific enzymes are 2-oxoglutarate:ferredoxin oxidoreductase (Oor), fumarate reductase (Frd) and ATP citrate lyase (Acl) (Fig. 2b). The stable carbon isotopic fractionation between CO_2 and biomass by the rTCA cycle is less than that by the CBB cycle, which is in the range 2–13‰ (House *et al*., 2003). Carbon isotopic studies and mRNA-based surveys have showed that the rTCA cycle may represent the principal carbon fixation pathway in deep-sea vent ecosystems (Van Dover & Fry, 1989; Van Dover *et al*., 2002; Campbell & Cary, 2004). The rTCA cycle-operating deep-sea vent chemoautotrophs include *Aquificae* (e.g. *Persephonella* and *Desulfurobacterium*; Zhang *et al*., 2002; Hügler *et al*., 2007) and *Epsilonproteobacteria* (e.g. *Hydrogenimonas* and *Sulfurovum*; Hügler *et al*., 2005; Takai *et al*., 2005; Nakagawa *et al*., 2007) (Table 1). These chemoautotrophs utilize similar metabolic strategies (described below), although *Aquificae* grow at higher temperatures (Nakagawa *et al*., 2003). Stable carbon isotopic analysis suggested that some hyperthermophilic deep-sea vent chemoautotrophs of *Desulfurococcales* (i.e. *Pyrodictium* and *Pyrolobus*) might operate the rTCA cycle (House *et al*., 2003). However, it has been reported that other chemoautotrophic *Desulfurococcales*, i.e. *Ignicoccus*, lack the key enzymes of the rTCA cycle (Hügler *et al*., 2003) and probably use an as yet uncharacterized CO_2 fixation pathway that starts from acetyl-CoA (Jahn *et al*., 2007). Currently unpublished genome sequences of chemoautotrophic *Desulfurococcales* would provide important clues in understanding their carbon fixation pathway (Table 1).

In comparison with deep-sea vent chemoautotrophs using the CBB cycle (described above), rTCA cycle chemoautotrophs inhabit more reducing transition zones between hydrothermal vents and low-temperature peripheral zones (Nakagawa *et al*., 2005a, b), potentially due to oxygen-sensitive enzymes involved in the rTCA cycle. Therefore, vent fauna hosting epsilonproteobacterial symbionts (mostly episymbionts) (e.g. alvinellid polychaete and alvinocarid shrimps) colonize close to vent emissions (Van Dover, 2000). Recently, the first rTCA cycle-based endosymbiosis between *Epsilonproteobacteria* (*Sulfurovum* sp. and *Sulfurimonas* sp.) and *Alviniconcha* gastropods were identified on the Central Indian Ridge (Kairei field) and in the south-west Pacific (PACMANUS field E and Vienna Woods of the Manus Basin, and STARMER II of North Fiji) (Fig. 1b) (Suzuki *et al*., 2005a, b; Urakawa *et al*., 2005). Interestingly, some *Alviniconcha* gastropods in the south-west Pacific (Lau Basin, Mariana backarc basin, PACMANUS field D of the Manus Basin, and White Lady of North Fiji) host not epsilon- but gammaproteobacterial endosymbionts using the CBB cycle to fix CO_2 (Fig. 1a) (Stein *et al*., 1988; Suzuki *et al*., 2005b), suggesting that the current symbiotic association between gastropods and chemoautotrophs results from independent and repeated symbiotic events. If this is correct, habitats for *Alviniconcha* gastropods hosting gammaproteobacterial endosymbionts might be more oxic than those for *Alviniconcha* gastropods hosting epsilonproteobacterial endosymbionts. Patterns in colonization of vent fauna and the distribution of carbon fixation pathways of endosymbionts may be relevant and should be further clarified.

3-HP and 3-HP/4-HB cycles

The 3-HP cycle was originally characterized in an anoxygenic, moderately thermophilic phototroph (i.e. *Chloroflexus*; Strauss & Fuchs, 1993). In this cycle, key enzymes are acetyl-CoA/propionyl-CoA carboxylase, malonyl-CoA reductase and propionyl-CoA synthase (Fig. 2c). Recently, the CO_2 fixation cycle of *M. sedula* was characterized in detail and designated the 3-HP/4-HB cycle (Berg *et al*., 2007). Most of the organisms using this pathway have the ability to grow heterotrophically. In this pathway, succinyl-CoA formed from acetyl-CoA and two CO_2 is converted to two acetyl-CoA molecules (Fig. 2c). The stable carbon isotopic fractionation between CO_2 and biomass by the 3-HP or 3-HP/4-HB cycle is similar to those by the rTCA

cycle, which is in the range 0.2–5.1‰ (House *et al.*, 2003). Although habitats for *Sulfolobales* are restricted to terrestrial acidic hot springs, key genes of the 3-HP/4-HB cycle, including 4-hydroxybutyryl-CoA dehydratase, were identified in the genome of marine group 1 *Crenarchaeota* (i.e. *Cenarchaeum symbiosum*; Hallam *et al.*, 2006) and in the Global Ocean Sampling (GOS) database (Rusch *et al.*, 2007; Yooseph *et al.*, 2007), pointing to the importance of this new pathway in global carbon cycling (Berg *et al.*, 2007). To date, none of the chemoautotrophs utilizing the 3-HP or 3-HP/4-HB cycles is endemic to deep-sea vents. Although *C. symbiosum* has a symbiotic association with a shallow-water sponge, *Axinella mexicana* (Preston *et al.*, 1996), none of the 3-HP or 3-HP/4-HB cycle-operating chemoautotrophs is yet shown in a symbiotic relationship with vent fauna (Table 1).

Reductive acetyl-CoA pathway

Unlike other CO_2 fixation pathways, this is a noncyclic pathway. The reductive acetyl-CoA pathway has been found only in chemoautotrophs, including a sulfate-reducing deltaproteobacterium (*Desulfobacterium autotrophicum*), acetogens (e.g. *Clostridium*) and methanogenic *Archaea* (e.g. *Methanobacterium* and *Methanosarcina*) (Meuer *et al.*, 2002; Ragsdale, 2004). These chemoautotrophs are strict anaerobes, as the reductive acetyl-CoA pathway involves enzymes highly sensitive to oxygen. In this pathway, one CO_2 is captured on a cofactor [tetrahydrofolate (THF) in acetogens; tetrahydromethanopterin (THMP) in methanogens] and reduced to a methyl group (Fig. 2d). The other CO_2 is reduced to a carbonyl group by the carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS) complex (Fig. 2d). The stable carbon isotopic fractionations between CO_2 and biomass by this pathway have been determined to be in the range 4.8–26.7‰ (House *et al.*, 2003). To our knowledge, deep-sea vent chemoautotrophs using this pathway are restricted to methanogens, including *Methanococcales* (e.g. *Methanocaldococcus* and *Methanothermococcus*) and *Methanopyrales* (i.e. *Methanopyrus*) (Table 1). These methanogens are widely distributed within mid-ocean ridge systems, backarc basin systems (Nakagawa & Takai, 2006; Takai *et al.*, 2006b) and even ridge flank systems (Nakagawa *et al.*, 2006). The reductive acetyl-CoA pathway has been of wide interest as it may represent the first microbial means of CO_2 fixation (Peretó *et al.*, 1999). Multidisciplinary lines of evidence have suggested that hyperthermophilic hydrogenotrophic methanogens may represent the most ancient microorganisms flourishing on the Archaean Earth (Takai *et al.*, 2006c). Stable isotopic analysis of CH_4 from fluid inclusions in Archaean hydrothermal precipitates has strongly suggested the emergence of methanogens at least 3.46 Ba (Ueno *et al.*, 2006). Recent genome analyses also supported the early emergence of methanogenic *Archaea* (3.8–4.1 Ba) (Battistuzzi *et al.*, 2004), of which sulfate-reducing *Archaeoglobus* is the closest living ancestor (Gao & Gupta, 2007). None of the reductive acetyl-CoA pathway using chemoautotrophs is yet known in any symbiotic relationship with vent fauna, although methanogenic endosymbionts of rumen ciliates have been well studied (Embley & Finlay, 1993).

Energy metabolism

Potential energy sources for deep-sea vent chemoautotrophy include reduced sulfur compounds, molecular hydrogen, reduced metals and ammonium. Concentrations of these potential energy sources are variable among hydrothermal systems but are often more than several millimolar (mM) in endmember vent fluids. The reductants are oxidized during the combined reduction of electron acceptors, including oxygen, nitrate, Fe(III), sulfur compounds, sulfate and CO_2. As microbial and faunal habitats in deep-sea vents occur in transition zones between reduced hydrothermal fluids and oxidized seawater, available redox couples and free energy are highly variable and are strongly controlled by the physical and chemical conditions of the habitats.

There have been some challenges to identify key factors controlling chemoautotrophic microbial community structures and their activities (Wilcock *et al.*, 2004). Thermodynamic calculation of the free energy change (ΔG) of redox reactions may provide a solution (McCollom & Shock, 1997; Shock & Holland, 2004; Tivey, 2004). One can predict the types of energy metabolisms in given habitats if physical and chemical conditions are measured (Shock & Holland, 2004). Nevertheless, the geochemical prediction of microbial metabolism still has substantial problems; kinetic parameters of energy metabolisms in a variety of chemoautotrophs are as yet unknown, and competitive and cooperative behaviors among different microorganisms (e.g. syntrophy between methanogens and bacteria) are not well considered (Takai *et al.*, 2006b). In addition, geochemical thermodynamic models have not been completely verified based on actual microbiological community structures and activities (Takai *et al.*, 2006b). Thus, patterns in chemoautotrophic microbial community structures and activities should be microbiologically clarified in more detail in various deep-sea vent environments and should be integrated into geochemical thermodynamic modeling. Here, we summarize typical energy metabolisms of deep-sea vent chemoautotrophs.

Sulfur oxidation

The microbial sulfur oxidation pathway has been studied intensively in anaerobic phototrophs (e.g. *Chlorobium* and *Allochromatium*), facultatively chemoautotrophic *Proteobacteria* (e.g. *Thiobacillus* and *Paracoccus*) and *Sulfolobales* (e.g. *Sulfolobus* and *Acidianus*) (Friedrich, 1998, 2005;

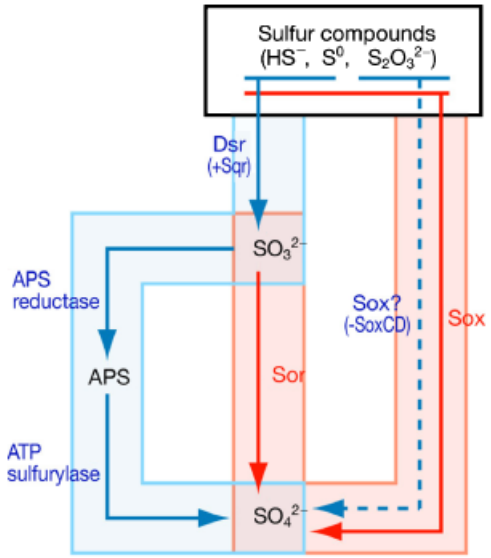

Fig. 3. Sox-dependent (shown in red) and Sox-independent (shown in blue) sulfur oxidation pathways of deep-sea vent chemoautotrophs. These pathways are used by *Epsilonproteobacteria* and *Gammaproteobacteria*, respectively.

Kletzin *et al.*, 2004). Sulfur oxidation pathways differ among sulfur oxidizers, but can be roughly classified into three different types based on the repertoire of sulfur-oxidizing enzymes: (1) the Sox-independent pathway of *Gammaproteobacteria* and anaerobic phototrophs, (2) the Sox-dependent pathway of *Alphaproteobacteria* and (3) the archaeal pathway (Fig. 3). Very little was known until recently about the sulfur oxidation pathway of deep-sea vent chemoautotrophs, although sulfur oxidation had been expected to be the primary energy metabolism driving deep-sea vent ecosystems. This was mostly due to the lack of pure cultures of the dominant sulfur oxidizers in deep-sea vents. Nevertheless, enzymatic assays on cells of sulfur-oxidizing endosymbionts physically separated from the host tissue have provided insights into their sulfur oxidation pathway. These assays revealed that gammaproteobacterial endosymbionts of tubeworms, mussels and clams have adenylylphosphosulfate reductase and ATP sulfurylase, indicating that they oxidize sulfite to sulfate via adenylylphosphosulfate (Nelson & Fisher, 1995). In addition, recent genomic analyses on the gammaproteobacterial endosymbionts of tubeworms (nearly completed genome) and clams (completed whole genomes) suggested the involvement of additional enzymes in the sulfur oxidation pathway, i.e. reversible dissimilatory sulfite reductase (Dsr), sulfide:quinone oxidoreductase (Sqr) and sulfur oxidation (Sox) multienzyme complex lacking *soxCD* (Fig. 3; Kuwahara *et al.*, 2007; Markert *et al.*, 2007; Newton *et al.*, 2007). These results suggest that gammaproteobacterial endosymbionts (at least clade I and clade III members) of vent fauna have the Sox-independent sulfur oxidation pathway, similar to those of other gammaproteobacterial sulfur oxidizers. Although the significance of Sox components in this type of sulfur oxidation pathway remains to be determined, the proteins may be responsible only for thiosulfate oxidation, as shown in *Allochromatium* (Fig. 3; Hensen *et al.*, 2006).

Recent successful isolation of deep-sea vent *Epsilonproteobacteria* has led to the detailed characterization of the sulfur oxidation pathway (Takai *et al.*, 2005; Nakagawa *et al.*, 2007). This group of bacteria accounts for a significant fraction of deep-sea vent chemoautotrophs (Takai *et al.*, 2004a, 2006b; Nakagawa *et al.*, 2005a, b; Huber *et al.*, 2007). Genomic analysis revealed that deep-sea vent *Epsilonproteobacteria* have the Sox-dependent sulfur-oxidizing pathway consisting of sulfite:cytochrome *c* oxidoreductase (Sor), Sqr and Sox multienzyme complex (Fig. 3). A total of 15 genes comprising a single *sox* gene cluster are identified for *Paracoccus pantotrophus* (Friedrich *et al.*, 2001). Of the *sox* genes, seven genes, *soxXYZABCD*, code for periplasmic proteins responsible for the oxidation of sulfur compounds (hydrogen sulfide, elemental sulfur, thiosulfate and sulfite) (Rother *et al.*, 2001). In deep-sea vent *Epsilonproteobacteria* genomes, most of the predicted *sox* genes formed two spatially separated gene clusters (Nakagawa *et al.*, 2007). Similarly, the organization of *sox* genes into separated clusters was reported in a deep-sea vent gammaproteobacterium *Thiomicrospira crunogena* (Scott *et al.*, 2006) and a nonvent epsilonproteobacterium *Sulfurimonas denitrificans* which was isolated from coastal marine sediments (Sievert *et al.*, 2008). The Sox components are also abundantly found in the GOS database, indicating that heterotrophic sulfur oxidation plays an important role in global sulfur cycling (Venter *et al.*, 2004). In addition to these genomics-based investigations, further biochemical studies of deep-sea vent chemoautotrophs are required to gain comprehensive understanding of the mechanisms of sulfur oxidation and its ecological significance.

Hydrogen oxidation

Until recently, hydrogen oxidation had been less well studied than sulfur oxidation, as molecular hydrogen is generally much less abundant than hydrogen sulfide in vent fluids (Charlou *et al.*, 2002). However, in some hydrothermal fields (e.g. ultramafic-hosted hydrothermal fields such as the Logatchev and Rainbow fields on the Mid-Atlantic Ridge), H_2 is the most dominant energy source in vent fluids (Charlou *et al.*, 2002; Takai *et al.*, 2006c). Hydrogenase is the enzyme that catalyzes the reversible oxidation of H_2 to protons and electrons. This enzyme is widely distributed among *Bacteria* and *Archaea* and is also found in some *Eukaryota*. Hydrogenases are classified into four different groups based on cellular function and amino acid

sequence: group 1, membrane-bound H_2-uptake hydrogenase; group 2, H_2-sensing or cyanobacterial hydrogenase; group 3, F_{420}-reducing, bifunctional hyperthermophilic hydrogenase, methylviologen-reducing hydrogenase and bidirectional NAD-linked hydrogenase; and group 4, membrane-bound H_2-evolving hydrogenase (Vignais *et al.*, 2001).

Chemoautotrophs with the ability to derive energy from hydrogen oxidation have been isolated from various deep-sea hydrothermal fields, including *Aquificales*, *Epsilonproteobacteria*, *Desulfurococcales*, *Methanococcales*, *Thermodesulfobacteriales* and *Deferribacterales* (Table 1). Analysis of deep-sea vent epsilonproteobacterial genomes revealed that they have unique sets of hydrogenases. *Sulfurovum* sp. NBC37-1 has four different sets of structural genes of hydrogenases, two of group 1, one of group 2 and one of group 4. *Nitratiruptor* sp. SB155-2 has three hydrogenases (one each of groups 1, 2 and 4) (Nakagawa *et al.*, 2007). Multiple hydrogenase gene clusters point to the importance of hydrogen oxidation for deep-sea vent chemoautotrophs.

In genomes of chemoautotrophic *Epsilonproteobacteria*, structural genes of group 2 hydrogenase are located immediately upstream of H_2-uptake hydrogenase structural genes (Nakagawa *et al.*, 2007; Sievert *et al.*, 2008). The group 2 hydrogenase and a histidine kinase act together as an H_2-signal transducer by controlling the phosphorylation state of transcription activators in *Alcaligenes* species (Lenz *et al.*, 1997). In contrast, the group 2 hydrogenase of *Aquifex aeolicus* is suggested to be involved in the carbon fixation pathway (Brugna-Guiral *et al.*, 2003). The physiological role of group 2 hydrogenase in *Epsilonproteobacteria* remains to be investigated (Sievert *et al.*, 2008). In addition, it seems paradoxical that hydrogen-oxidizing deep-sea vent *Epsilonproteobacteria* have a putative gene cluster of H_2-evolving hydrogenase (group 4), which is also referred to as *Escherichia coli* hydrogenase 3 (Hyc) or hydrogenase 4 (Hyf)-type hydrogenase. The catalytic subunit of these hydrogenases is oriented towards the cytoplasmic site. When *E. coli* is grown under anaerobic conditions, the *E. coli* hydrogenase 3 and 4, together with formate dehydrogenase (Fdh), couples the oxidation of formate with the reduction of protons. However, only genes encoding the alpha subunit of Fdh were identified in both deep-sea vent *Epsilonproteobacteria* genomes. Other examples of H_2-evolving hydrogenase have been reported for a few groups of microorganisms, including methanogens, *Rhodospirillum rubrum*, and hyperthermophilic fermentative archaea (Silva *et al.*, 2000). It is suggested that the H_2-evolving hydrogenase of methanogens acts as a proton pump involved in the conversion of the carbonyl group of acetate to CO_2 and H_2 (Künkel *et al.*, 1998). The expression of H_2-evolving hydrogenase of *R. rubrum* is induced by carbon monoxide. This hydrogenase, together with CO dehydrogenase, catalyzes the oxidation of CO to CO_2 and H_2 with energy recovery (Fox *et al.*, 1996). The CO dehydrogenase was identified in neither of the deep-sea vent *Epsilonproteobacteria* genomes. In a hyperthermophilic fermentative archaeon, *Pyrococcus furiosus*, group 4 hydrogenases produce H_2 via electrons carried by ferredoxin, which is directly coupled the ATP synthesis by means of a proton motive force (Sapra *et al.*, 2003). As the growth characteristics of deep-sea vent *Epsilonproteobacteria* are different from those of microorganisms possessing previously characterized H_2-evolving hydrogenases, the physiological function of this enzyme remains to be investigated. Nevertheless, the presence of this enzyme is suggestive of efficient energy metabolism similar to the 'intracellular H_2-cycling mechanism' of sulfate-reducing bacteria (Odom & Peck, 1981), in which the H_2 produced by H_2-evolving hydrogenases diffuses across the membrane, and is then oxidized by periplasmic H_2-uptake H_2ases to form a proton gradient.

Metal oxidation

Vent fluids are enriched in reduced metals (Fe, Mn, As, Cu, etc.) with Fe(II) as the most dominant metal (several mM in many vent fluids) (Charlou *et al.*, 2002). In agreement with the metal-rich niche, deep-sea vent *Epsilonproteobacteria* have detoxification mechanisms of a wide array of heavy metals (Nakagawa *et al.*, 2007). However, very little is known about energy-yielding metal oxidation in deep-sea vents. A 'zetaproteobacterium' *M. ferooxydans* from Loihi Seamount, and several *Alphaproteobacteria* strains are the only chemoautotrophic Fe(II) oxidizers described from deep-sea vents (Table 1; Edwards *et al.*, 2003; Emerson *et al.*, 2007). Manganese(II) oxidation mediated by heterotrophic *Bacillus* species in hydrothermal plumes was reported from the Guaymas Basin hydrothermal field (Dick *et al.*, 2006). At present, it is assumed that Mn(II) oxidation in deep-sea vents may be not substantially catalyzed by chemoautotrophs (Dick *et al.*, 2006). However, as the isolation of *M. ferooxydans* renewed the ecological significance of chemoautotrophic Fe(II) oxidation, future discoveries may provide new insights into chemoautotrophic Mn(II) oxidation.

Ammonia oxidation

Abundant ammonium (up to several millimolar in vent fluids) is produced by thermal degradation of organic compounds in deep-sea hydrothermal fields, especially in sediment-hosted hydrothermal fields such as the Guaymas Basin, Juan de Fuca Ridge and Okinawa Trough Backarc Basin. It was demonstrated in the hydrothermal plume of the Juan de Fuca Ridge that ammonium was rapidly consumed by chemoautotrophs once it was discharged from vents to ambient seawater (Lam *et al.*, 2004). Although none of the ammonia-oxidizing chemoautotrophs has yet been

isolated from deep-sea vents, it is assumed that marine group 1 *Crenarchaeota* dominating in the vicinity of deep-sea vents play a significant role in ammonia oxidation (Takai *et al*., 2004b). Besides marine group 1 *Crenarchaeota*, a diversity of as yet uncultivated ammonium-oxidizing bacteria and archaea may be involved in chemoautotrophic primary production in deep-sea vents.

Concluding remarks

Genomic technologies have offered unprecedented opportunities to achieve comprehensive understanding of the molecular mechanisms of deep-sea vent chemoautotrophy. In addition to the existing Sanger sequencing method, the recent introduction of the pyrosequencing platform offers a promising high-throughput sequencing technology (Margulies *et al*., 2005). Among deep-sea vent chemoautotrophs, symbionts of vent fauna have become the major targets of whole genome sequencing efforts, as symbiont genomes may provide insight into the molecular mechanisms that underpin symbiont–host interactions. However, in order to clarify the molecular interactions between symbionts and hosts, further efforts would be required to sequence host vent fauna genomes. One problem here is that functionally characterized gene sequences are rare for deep-sea vent fauna. At the time of writing, the GOLD database (http://www.genomesonline.org) lists only two ongoing expression sequence tag projects on vent fauna (*Alvinella* and *Riftia*). As candidate molecular mechanisms that support symbiotic relationships, deep-sea vent *Epsilonproteobacteria* were demonstrated to have the evolutionary precursors of virulence factors of pathogenic *Epsilonproteobacteria* such as an *N*-linked glycosylation system, by which pathogenic *Campylobacter* adhere to and invade host epithelia (Nakagawa *et al*., 2007). In contrast, very little is yet known about the molecular interactions between vent fauna and gammaproteobacterial symbionts. As with deep-sea vent *Epsilonproteobacteria*, gammaproteobacterial symbionts have pathogenic relatives (e.g. endosymbionts of *Bathymodiolus/Calyptogena* are closely related to highly pathogenic *Francisella tularensis*). Establishing a symbiotic relationship (whether endosymbiotic or episymbiotic) with an animal should provide more opportunity to interact with other microbes, including pathogens to the host, thus leading to the acquisition of virulence genes in pathogenic descendants (Nakagawa *et al*., 2007). Further genomic studies would clarify the previously unrecognized evolutionary links between deep-sea vent symbionts and human/animal pathogens.

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