Deep-sea vent chemoautotrophs: diversity, biochemistry and ecological significance
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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., H₂, H₂S, CO and CO₂, 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 carboxydrotrophy 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 H₂ 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.
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 epibionts belong to the Gammaproteobacteria (Fig. 1a) and Epsilonproteobacteria (Fig. 1b), respectively. Although close relatives of epsilonproteobacterial symbionts (both epibionts and endosymbionts) are now culturable, gammaproteobacterial chemoautotrophs isolated from
deep-sea vents are only distantly related to any chemoauto-
trophic symbionts (Fig. 1a). The unculturable gammapro-
tebacterial 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 CO2 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 CO2 differ among pathways (Fig. 2). The CBB cycle requires three molecules of ATP for the fixation of one CO2, while the rTCA cycle requires one ATP for one CO2. In the reductive acetyl-CoA pathway, used by chemoautotrophs inhabiting H2-rich environments, CO2 is directly reduced by H2 and thus ATP input is not required. Chemoautotrophs may have evolved to adopt the most energetically parsimonious CO2 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 CO2 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 CO2 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 CO2 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 CO2 or O2 (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 (L8S8). Form II RuBisCO, a dimer of large subunits (L2), has a lower specificity for CO2 and thus is more effective under CO2-rich conditions (Tabita et al., 2007). The RuBisCOs, ...
<table>
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<tr>
<th>Phylogenetic group</th>
<th>Growth temperature range of isolates (°C)</th>
<th>CO₂ fixation pathway</th>
<th>Energy source</th>
<th>Major habitat</th>
<th>Host animal (type of symbiosis; bacteriocyte)</th>
<th>Relationship to oxygen</th>
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<td>rTCA</td>
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<td>Mixing zone</td>
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<td>Ferrous iron</td>
<td>Metalliferous deposit</td>
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<td>Chimney structure</td>
<td>Bresilioid shrimps (Episymbiosis?, gut)</td>
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<td>Ammonium</td>
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<td>rTCA variant?</td>
<td>Hydrogen</td>
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<td>Hydrogen</td>
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<td>Methanopryri (Methanopyrus*)</td>
<td>84–110</td>
<td>reductive</td>
<td>Hydrogen</td>
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^For which the genome sequence is available or being sequenced.

^Abundantly found in tubes of alvinellid polychaetes Nakagawa et al. (2005b).
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 *Beggioia*; 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 CO₂ 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 13C-enriched biomass of Ridgeiidae (e.g. *Riftia* and *Ridgeia*) and Tevniaidae (e.g. *Tevnia* and *Oasisia*) tubeworms (δ13C 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 CO₂ 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 kodakarenensis 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 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 archaean (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. Desulfovibrio 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 CO₂ 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 chemosynthetic trophs include Aquificales (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 Aquificales grow at higher temperatures (Nakagawa et al., 2003). Stable carbon isotopic analysis suggested that some hyperthermophilic deep-sea vent chemoautotrophs of Desulfurococcaceae (i.e. Pyrodictium and Pyrolobus) might operate the rTCA cycle (House et al., 2003). However, it has been reported that other chemoautotrophic Desulfurococcus, i.e. Ignicoccus, lack the key enzymes of the rTCA cycle (Hügler et al., 2003) and probably use an as yet uncharacterized CO₂ fixation pathway that starts from acetyl-CoA (Jahn et al., 2007). Currently unpublished genome sequences of chemoautotrophic Desulfurococcaceae 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 chemosynthetic trophs 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 epsiprobacters) (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 CO₂ (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 toxic 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 CO₂ 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 CO₂ is converted to two acetyl-CoA molecules (Fig. 2c). The stable carbon isotopic fractionation between CO₂ 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. Crenarchaeum 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 chemosauotrophs 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 chemosauotrophs is yet shown in a symbiotic relationship with vent fauna (Table 1).

Reductive acetyl-CoA pathway

Unlike other CO₂ fixation pathways, this is a noncyclic pathway. The reductive acetyl-CoA pathway has been found only in chemosauotrophs, including a sulfate-reducing deltaproteobacterium (Desulfbacterium autotrophicum), acetogens (e.g. Clostridium) and methanogenic Archaea (e.g. Methanobacterium and Methanosarcina) (Meuer et al., 2002; Ragdale, 2004). These chemosauotrophs are strict anaerobes, as the reductive acetyl-CoA pathway involves enzymes highly sensitive to oxygen. In this pathway, one CO₂ is captured on a cofactor [tetrahydrofolate (THF) in acetogens; tetrahydromethanopterin (THMP) in methanogens] and reduced to a methyl group (Fig. 2d). The other CO₂ 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 CO₂ and biomass by this pathway have been determined to (Fig. 2d). The stable carbon isotopic fractionations between CO₂ and biomass by this pathway have been determined to (Fig. 2d). The stable carbon isotopic fractionations between CO₂ and biomass by this pathway have been determined to (Fig. 2d). The stable carbon isotopic fractionations between CO₂ and biomass by this pathway have been determined to (Fig. 2d). The stable carbon isotopic fractionations between CO₂ and biomass by this pathway have been determined to (Fig. 2d). The stable carbon isotopic fractionations between CO₂ and biomass by this pathway have been determined to

Energy metabolism

Potential energy sources for deep-sea vent chemosauotrophy 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 CO₂. 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 chemosauotrophic microbial community structures and their activities (Wilcock et al., 2004). Thermodynamic calculation of the free energy change (∆G) 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 chemosauotrophs 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 chemosauotrophic 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 chemosauotrophs.

Sulfur oxidation

The microbial sulfur oxidation pathway has been studied intensively in anaerobic phototrophs (e.g. Chlorobium and Allochromatium), facultatively chemosauotrophic Proteobacteria (e.g. Thiobacillus and Paracoccus) and Sulfolobales (e.g. Sulfolobus and Acidianus) (Friedrich, 1998, 2005; 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 chemosauotrophs is yet known in any symbiotic relationship with vent fauna, although methanogenic endosymbionts of rumen ciliates have been well studied (Embley & Finlay, 1993).
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-dependent pathway of Gammaproteobacteria and Epsilonproteobacteria 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), sulfidequinone 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), H₂ 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 H₂ 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 H₂-uptake hydrogenase; group 2, H₂-sensing or cyanobacterial hydrogenase; group 3, F₄₃₀-reducing, bifunctional hyperthermophilic hydrogenase, methylviologen-reducing hydrogenase and bidirectional NAD-linked hydrogenase; and group 4, membrane-bound H₂-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 Deferrribacterales (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 2, 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 H₂-uptake hydrogenase structural genes (Nakagawa et al., 2007; Sievert et al., 2008). The group 2 hydrogenase and a histidine kinase act together as an H₂-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 H₂-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 H₂-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 H₂-evolving hydrogenase of methanogens acts as a proton pump involved in the conversion of the carbonyl group of acetate to CO₂ and H₂ (Künkelt et al., 1998). The expression of H₂-evolving hydrogenase of *R. rubrum* is induced by carbon monoxide. This hydrogenase, together with CO dehydrogenase, catalyzes the oxidation of CO to CO₂ and H₂ 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 H₂ 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 H₂-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 H₂-cycling mechanism’ of sulfate-reducing bacteria (Odom & Peck, 1981), in which the H₂ produced by H₂-evolving hydrogenases diffuses across the membrane, and is then oxidized by periplasmic H₂-uptake H₂ases 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 chemoaotrophic 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 chemolithoautotrophic 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 chemosynthesis. 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 chemosynthetic symbionts, 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/Calypogena are closely related to highly pathogenic Francisella tularensis). Establishing a symbiotic relationship (whether endosymbiotic or epibiotic) 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.

**References**


