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The influence of ultramafic rocks on microbial communities at the Logatchev hydrothermal field, located 15°N on the Mid-Atlantic Ridge

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Abstract

The ultramafic-hosted Logatchev hydrothermal field (LHF) on the Mid-Atlantic Ridge is characterized by high hydrogen and methane contents in the subseafloor, which support a specialized microbial community of phylogenetically diverse, hydrogen-oxidizing chemolithoautotrophs. We compared the prokaryotic communities of three sites located in the LHF and encountered a predominance of archaeal sequences affiliated with methanogenic *Methanococcales* at all three. However, the bacterial composition varied in accordance with differences in fluid chemistry between the three sites investigated. An increase in hydrogen seemed to coincide with the diversification of hydrogen-oxidizing bacteria. This might indicate that the host rock indirectly selects this specific group of bacteria. However, next to hydrogen availability further factors are evident (e.g. mixing of hot reduced hydrothermal fluids with cold oxygenated seawater), which have a significant impact on the distribution of microorganisms.

Introduction

Deep-sea hydrothermal environments contain a variety of biotopes which are characterized by steep physical and chemical gradients (Kelley *et al*., 2002). The physico-chemical conditions which provide the essentials for microbial life include pH, temperature, oxygen levels and energy sources, e.g. hydrogen, sulfur and methane (Kelley *et al*., 2002). The most important physiological group of microorganisms is the chemolithoautotrophs. They are responsible for the local microbial primary production.

Both basalt- and ultramafic-hosted hydrothermal systems are found in Mid-Ocean Ridge spreading areas. The type of host rock determines the chemical composition of the fluids emitted from the vent systems. In turn, these fluid emissions supply the indigenous prokaryotes with the energy and carbon sources necessary to fuel primary production. The fluids found in ultramafic-hosted systems reveal significantly higher concentrations of hydrogen than those which occur in basalt-hosted systems (Wetzel & Shock, 2000).

Several studies are available on the microbial diversity of geographically distinct basalt-hosted systems. In contrast, only three (including the present study) have concerned themselves with ultramafic-hosted hydrothermal environments (the Lost City, Rainbow and Logatchev hydrothermal fields; for references see Table 1). Generally, the chemolithoautotrophic communities of hydrothermal environments show a clear predominance of only a few phylogenetic lineages. These include representatives of *Methanococcales*, *Epsilonproteobacteria* or *Aquificales* (e.g. Huber *et al*., 2002, 2003; Takai *et al*., 2003, 2004a; Nakagawa *et al*., 2005a).

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Table 1. Fluid physico-chemical parameters and selected microorganisms at different hydrothermal vents

Geological setting	Basaltic		Ultramafic				
Mid-Ocean Ridge	EPR	CIR		MAR			
Hydrothermal vent field	NT[†]	Edmond[‡]	Rainbow[§]	Logatchev[¶]			Lost City[‖]
Vent site of microbiological sample	Near Bio 9	Fuzzy toothpick		Irina II	Irina I	Site B	Atlantis massif
Physico-chemical parameter							
T (°C)	350	70	360*	170	300–350		40–91
pH (25 °C)	ND	ND	2.9–3.1*	7.3	6.2	3.8	9.0–11.0
H_2S (μmol kg^{-1})	≤ 3.300*	ND	≤ 2.500	116	277	1.241	64*
H_2 (mmol kg^{-1})	≤ 0.3*	0.2*	13.0*	2.2	5.9	1.8	≤ 14
CH_4 (mmol kg^{-1})	≤ 0.015*	0.4*	2.2*	0.7	1.5	0.6	≤ 2
Bacteria							
Gammaproteobacteria							
Thiomicrospira	−	−	−	−	−	−	+
Methylococcales	−	−	−	−	−	−	+
Deltaproteobacteria	+	−	+	+	−	+	+
Epsilonproteobacteria							
Group A	−	−	−	+	−	−	−
Group B	+	+	+	+	+	+	−
Group D	−	+	−	−	+	−	−
Group F	+	+	+	+	+	+	+
Group G	−	−	−	+	−	−	−
Sulfurospirillum	+	+	−	−	+	−	+
Aquificales	+	+	−	−	+	−	−
Desulfurobacterium	−	−	−	−	+	−	−
Archaea							
Methanococcales	+	−	+	+	+	+	−
Methanosarcinales ANME Group	−	−	ND	−	−	+	+

[†]Fluid chemical parameters (Von Damm & Lilley, 2004), temperature and microbiology data (Kormas *et al.*, 2006).
[‡]Fluid chemical parameters (Van Dover *et al.*, 2001), temperature and microbiology data (Hoek *et al.*, 2003).
[§]Fluid physico-chemical parameters (Donval *et al.*, 1997), microbiology data (López-García *et al.*, 2003; Nercessian *et al.*, 2005).
[¶]Temperature measurements (Lackschewitz *et al.*, 2005), fluid chemical parameters and microbiology data (the present study), H_2S, H_2 and CH_4 concentrations are minimum values.
[‖]Fluid physico-chemical parameters (Kelley *et al.*, 2001) and microbiology data (Schrenk *et al.*, 2004; Brazelton *et al.*, 2006).
Fluid data not obtained from identical microbiological sampling sites are marked by '*'. All chemical compositions of vent fluids are end-member concentrations. EPR, CIR and MAR denote East Pacific Rise, Central Indian Ridge and Mid-Atlantic Ridge respectively; NT denotes Northern Transect; ND corresponds to not determined; microorganisms detected/not detected are indicated by a '+ or −'.

Members of the archaeal order *Methanococcales* are strictly anaerobic and use hydrogen and carbon dioxide as a substrate for methanogenesis (Whitman *et al.*, 1992). They have been associated with the subvent biosphere (Huber *et al.*, 2002; Nakagawa *et al.*, 2005a), where they are probably among the most important primary producers (Huber *et al.*, 2002). Within the bacterial domain many affiliates of the *Epsilonproteobacteria* have been described as autotrophs (Campbell *et al.*, 2006). They might also play a considerable part in primary production in vent environments. Their ability to utilize a wide range of electron donors and electron acceptors (e.g. hydrogen, sulfur compounds, nitrate and oxygen) suggests their importance in hydrogen, sulfur and nitrogen cycling in hydrothermal biotopes (Takai *et al.*, 2003; Nakagawa *et al.*, 2005b; Campbell *et al.*, 2006). Epsilonproteobacterial representatives have been classified into at least six subgroups, known as A, B, C, D, F and G (Corre *et al.*, 2001). However, it recently came to light that the group C sequences had been misclassified (Campbell *et al.*, 2006). Other chemolithotrophic bacteria involved in hydrogen oxidation include thermophiles of deeply rooted lineages e.g. *Persephonella* spp. or *Desulfurobacterium* spp. (L'Haridon *et al.*, 1998; Götz *et al.*, 2002). Additionally, sulfur-oxidizing mesophiles such as *Thiomicrospira* spp. have been encountered in hydrothermal emissions (Jannasch *et al.*, 1985; Takai *et al.*, 2004b).

This is the first assessment of microorganisms inhabiting the Logatchev hydrothermal field (LHF). The aim of this study was to evaluate the microbial communities present in hydrothermal vent emissions at three sites and to identify possible shifts in community composition linked to changes in the chemistry of the hydrothermal fluids.

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Ultramafic-hosted hydrothermal systems possibly represent our closest analogue to early earth environments (Holm & Charlou, 2001). They could have played a role in the origin and evolution of life (Shock & Schulte, 1998). It is therefore vital that we expand our understanding of the interplay between source rock, fluid chemistry and the microbial populations in ultramafic-hosted hydrothermal systems such as the LHF.

Materials and methods

Site description, sample collection and fluid characteristics

Hydrothermal fluid samples were retrieved during dives made by the remotely operated vehicle (ROV) QUEST (MARUM, University of Bremen) during the HYDROMAR I (M60/3, 2004) and HYDROMAR II (M64/2, 2005) cruises to the LHF. The LHF is located at 14°45′N and 44°58′W on the northern Mid-Atlantic Ridge (MAR) in water depths of between 2960 and 3060 m (Bogdanov *et al.*, 1997; Kuhn *et al.*, 2004). High-temperature fluid emissions were collected from two smoking craters (Irina I and Site B) and the chimney structure at Irina II, which are all located within an area of 0.04 km^2.

The samples were retrieved using a pumped flow-through system (Kiel Pumping System KIPS) specially designed for the ROV QUEST (Garbe-Schönberg *et al.*, 2006). To minimize microbial cross-contamination between the ambient seawater and the hydrothermal fluids the nozzle of the KIPS was placed into the hot vent orifice before collection of the samples. To ensure a complete exchange of fluids while samples were being taken, pumping continued for *c.* 1 h. Fluid chemical analysis and microbial diversity studies were conducted using hydrothermal liquids from aliquots of the same sample. Once on board the ship, the liquids intended for microbiological studies were concentrated on 0.2-μm pore size polycarbonate filters (Sartorius) and stored at − 20 °C.

While exit temperatures of 300–350 °C were measured in fluid emissions from the smoking crater sites temperatures recorded for fluid outflow at the main chimney complex (Irina II) were lower (170 °C) (Table 1) (Lackschewitz *et al.*, 2005). The LHF ultramafic-hosted hydrothermal system shows distinct differences in rock mineralogy (Kuhn *et al.*, 2004). The reaction of heated seawater with gabbroic rocks is indicated. Serpentinization processes, caused by ultramafic rock-water interactions, are responsible for extremely high hydrogen ($\leq$ 19 mM) and methane ($\leq$ 3.5 mM) concentrations in the hydrothermal fluids, while sulfide concentrations do not exceed 3.5 mM (Schmidt *et al.*, 2007).

Analysis of fluid chemical parameters

The pH and sulfide concentrations were determined immediately after sample recovery. The pH was measured (Mettler electrodes with Ag/AgCl reference electrode) at 25 °C in unfiltered sample aliquots. Sulfide concentrations were determined photometrically following the methylene blue method (Cline, 1969) or, for samples with low concentrations, by voltammetry (Metrohm Application Bulletin 199/3e). Methane was analyzed on board by applying a purge and trap technique (Seifert *et al.*, 1999). In order to determine the $\delta^{13}C$ of methane, the water samples were degassed into a high-grade vacuum. Aliquots of the released gas were stored in gastight glass ampoules for later on-shore analysis by GC-Isotope-Ratio-Mass-Spectrometry (Seifert *et al.*, 2006). For on-board measurements of dissolved hydrogen the water sample was degassed into a high-grade vacuum. Aliquots of the released gas were analyzed by gas chromatography (Thermo Electron Corporation Trace GC Ultra, column: packed stainless steel (Molecular sieve 5 Å), oven temperature: isotherm 40 °C, carrier gas: He, detection: pulsed discharge detector). Analytical procedures were calibrated daily with standard commercial gas (LINDE). Extrapolation of the sample concentrations to end-member concentration was carried out on the assumption that the hydrothermal end-member fluids do not contain dissolved magnesium ($Mg = 0$) (Mottl & Holland, 1978). All presented values are end-member concentrations.

DNA extraction, 16S rRNA gene amplification, cloning and sequencing

DNA was extracted from filters using the Ultra Clean Soil DNA Isolation Kit (MoBio) according to the manufacturer's instructions. Archaeal and bacterial 16S rRNA genes were PCR-amplified using oligonucleotide primer sets consisting of 21F and 958R (DeLong, 1992) and 27F and 1492R (Lane, 1991), respectively. Primers (50 pmol μL^{-1}), 1 μL (bacteria) and 1.5 μL (archaea) of DNA template, and sterile water were added to PuReTaq Ready-To-Go-PCR Beads (Amersham Biosciences) to a total volume of 25 μL. An initial denaturation step (92 °C for 2 min) was followed by 20 cycles of 92 °C for 40 s, 50 °C for 40 s, 72 °C for 1 min for bacteria and 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min for archaea. The final extension was 5 min at 72 °C. To minimize PCR bias 20 cycles were conducted (Qiu *et al.*, 2001). The amplified product was purified by the Roche PCR purification kit according to the manufacturer's instructions and reamplified as described above using 1 μL of the purified extracts. PCR products were repurified as mentioned previously and subcloned with a TOPO-TA cloning kit (Invitrogen, Carlsbad, CA). In order to screen for 16S rRNA genes, 100 clones for each site were randomly picked and then resuspended in 25 μL of sterile water. The

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clones were checked for correct insert size by PCR with the M13F and M13R vector primers. PCR products of the correct size (~1500 bp) were screened and partially sequenced (~500 bp) for bacteria or archaea with 27F or 21F, respectively. Sequencing was performed using the ABI PRISM® BigDye™ Terminator Ready Reaction Kit (Applied Biosystems) and an ABI PRISM® 310 Genetic Analyzer (Perkin Elmer Applied Biosystems). To clarify phylogenetic affiliation of specific bacterial sequences, full sequences were obtained using the primers 342F, 1492R (Lane, 1991), 534R (Muyzer *et al.*, 1993) and 1094R (Munson *et al.*, 1991).

Phylogenetic analysis

Sequences were edited and assembled with Lasergene Software SEQMAN (DNAStar Inc.). Chimeric sequences were identified using the CHIMERA-CHECK software available from Ribosomal Database Project (Cole *et al.*, 2003) and then eliminated. Sequences were compared with DNA sequences in the public domain through BLASTN searches (Altschul *et al.*, 1997). Sequence data was compiled using ARB software (www.arb-home.de) and then aligned with sequences obtained from the GenBank database using the ARB FASTALIGNER utility (Ludwig *et al.*, 2004). The resulting alignments were manually verified against known secondary structure regions. Maximum-likelihood-based trees were constructed using PhyML (Guindon & Gascuel, 2003). The PHYLIP version 3.65 package (J. Felsenstein, University of Washington, Seattle) was used additionally to construct a maximum-parsimony tree (DNAPARS) for sequences of deeply rooted lineages. Bootstrap analysis (SEQBOOT) was used to provide confidence estimates for maximum parsimony tree topologies. All trees were constructed using 100 bootstrap replicates and near full-length sequences. The trees were imported into ARB and shorter sequences added to trees using the Parsimony Quick and Add option.

Nucleotide sequence accession numbers

All 16S rRNA gene sequences obtained in this study were submitted to DDBJ/EMBL/GenBank database and assigned the accession numbers AM268531–AM268882, AM279649 and AM279650.

Results

Fluid Chemistry

End-member concentrations of the hydrothermal fluids emanating at the three vent sites (Irina I, Irina II and Site B) are summarized in Table 1. Both the pH measurements and sulfide concentrations of Irina I and Irina II were strongly affected by dilution of the hydrothermal sample with seawater prior to and during sampling (Mg > 40 mM). The pH (25 °C) for fluids at Irina I, Irina II and Site B were 6.2, 7.3 and 3.8, respectively (Table 1). Fluid samples taken at Irina I revealed the highest hydrogen and methane concentrations in this study (5.9 and 1.5 mM, respectively), but low sulfide contents (277 μM) (Table 1). The hydrogen and methane contents of fluid samples taken at Irina II amounted to 2.2 and 0.7 mM, respectively, and 116 μM of sulfide was found (Table 1). In contrast, at Site B, where the level of seawater dilution was lowest (50% end-member), the highest sulfide concentrations in this study were observed (1.2 mM). Hydrogen and methane contents accounted for 1.8 and 0.6 mM, respectively. Stable carbon isotope signatures were determined for methane and ranged between − 9.3‰ and − 13.9‰.

Phylogenetic analysis

Three archaeal and bacterial clone libraries were constructed with samples originating from three vent sites in the ultramafic-hosted LHF. Fluid emissions from the chimney structure at Irina II and the smoking craters at Irina I and Site B yielded 65, 80 and 93 bacterial and 46, 35 and 34 archaeal sequences, respectively. Clone sequences with similarities of ≥97% were defined as an operational taxonomic unit (OTU).

Sequences retrieved from fluid emissions at the LHF, but which are generally found in the open water column (e.g. Acinas *et al.*, 1999; Long & Azam, 2001; Bano & Hollibaugh, 2002), were not taken into consideration. Their presence was assumed to be caused by mixing with ambient seawater during collection of fluid samples, with the ultramafic setting of the LHF not being a significant selection factor. Bacterial sequences not considered for this reason amount to 73%, 30% and 32% of fluid emissions at Irina I, Irina II and Site B, respectively. These mainly include *Gammaproteobacteria* (e.g. *Vibrionales, Alteromonadales*, *Oceanospirillales*), *Alphaproteobacteria* (*Rhodobacterales*) and *Bacteroidetes* as well as very few *Betaproteobacteria* (*Burkholderiales*), *Planctomycetales*, *Clostridia* or *Actinobacteria*. Among archaea, 26%, 33% and 47% of sequences retrieved from fluids at Irina I, Irina II and Site B, respectively, were excluded from analyses for the same reason as that mentioned above. This includes a major faction affiliated to the Crenarchaeotic Marine Benthic Group I, which occurs at various locations. However, percentages of OTUs were calculated from the total number of all bacterial or archaeal clone sequences obtained per vent site.

A large part of the indigenous microbial community (bacteria and archaea) of the LHF was related to organisms known as autotrophic hydrogen-oxidizers. The limitations to inferring physiological properties from the analysis of 16S rRNA gene sequences have already been demonstrated (e.g.

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Kashefi *et al*., 2002). Nevertheless, in some instances, it may be possible to infer physiological traits of uncultured organisms from the physiology of very closely related microorganisms that are available in culture or from group-specific characteristics. For example, all cultured *Epsilonproteobacteria* affiliated to group D are capable of oxidizing hydrogen (for review see Campbell *et al*., 2006).

Epsilonproteobacteria

At Irina I, Irina II and Site B affiliates of the *Epsilonproteobacteria* constituted 19%, 45% and 49%, respectively, of all retrieved bacterial clone sequences. Phylogenetically diverse representatives of *Epsilonproteobacteria* were encountered in emissions at Irina I and Irina II (Fig. 1a). This included members of groups B, D, F and *Sulfurospirillum* spp. (Irina I) and affiliates of groups A, B, F and G (Irina II) (Fig. 1a). By contrast, Site B epsilonproteobacterial sequences were restricted to groups B and F (Fig. 1a).

At Irina II, representatives of group A comprised 9% of the clone sequences and were closely related (99%) to *Hydrogenimonas thermophila* (Fig. 1a). At Irina I only 6% of bacterial sequences were related to members of group F. In contrast, they contributed significantly to the clone libraries recovered from fluids at Irina II and Site B (31% and 47%, respectively). The majority of these sequences clustered into a deeply diverging group with no known cultured representatives (Fig. 1a). They were related exclusively to sequences originating from vent environments. The closest described isolate of these LHF sequences was *Sulfurovum lithotrophicum* (sequence similarities 86–98%). Only a minority of clone sequences at Irina I (6%), Irina II (3%) and Site B (1%) were associated with group B (Fig. 1a). Sequences affiliated to *Sulfurospirillum* spp. were restricted to Irina I (Fig. 1a).

Deeply rooted lineages

Sequences of deeply rooted lineages constituted a minor fraction of clones (Fig. 1b). At Irina I, 5% of the bacterial clone sequences were closely related to *Desulfurobacterium* sp. (99%), *Persephonella* sp. (98%) and *Oceanithermus profundus* (99%) (Fig. 1b).

A single sequence originating from fluids emitted at Irina I and 9% of sequences at Irina II were affiliated with sequences of the group C *Epsilonproteobacteria*, recently recognized as having been misclassified (Campbell *et al*., 2006). New tree calculations place them as a new group (RE1) in close proximity to the candidate division SR1 (Fig. 1b). High bootstrap values support this position in maximum likelihood and maximum parsimony tree topologies (Fig. 1b). The OTUs were related to sequences from different hydrothermal environments such as the Guaymas Basin (Dhillon *et al*., 2003), the East Pacific Rise (Alain *et al*., 2004) or the Mid-Atlantic Ridge (Corre *et al*., 2001) (Fig. 1b). Two sequences from Site B were grouped in the uncultured candidate divisions SR1 and OD1, which were only distantly related (94%) to their closest relatives (Fig. 1b).

Gammaproteobacteria

At Irina II 8% of the bacterial sequences were identical to thioautotrophic and methylotrophic symbionts of *Bathymodiolus* spp. A single sequence at Irina I clustered with methylotrophic symbionts of these vent mussels. In contrast, at Site B no sequences were related to symbionts of *Bathymodiolus* spp. However, at this site two gammaproteobacterial sequences were found that resembled symbiont sequences of *Escarpia spicata* (Di Meo *et al*., 2000) and *Codakia orbicularis* (Gros *et al*., 1996).

Deltaproteobacteria

Fluids at Irina II and Site B additionally included members of the *Deltaproteobacteria* (8% and 15%, respectively). They were exclusively associated with the *Desulfobulbaceae* family (Fig. 1c). The majority of fluid sequences at Irina II (5%) and at Site B (11%) were related to *Desulfocapsa sulfexigens* (Fig. 1c).

Archaea

At the Irina I, Irina II and Site B vent locations, members of the order *Methanococcales* accounted for 74%, 68% and 50% of archaeal clone sequences, respectively (Fig. 1d). Only a few species of the Deep-Sea Hydrothermal Vent Euryarchotic Groups I and II, with no cultured representatives, were identified (data not shown).

Sequences related to "*Methanococcus aeolicus*" prevailed among the archaeal clone libraries at Irina I (43%), Irina II (54%) and Site B (47%) (Fig. 1d). Representatives related to *Methanocaldococcus infernus* were also present at Irina I (14%), at Irina II (4%) and at Site B (3%) (98–99% sequence similarities). Other methanogens found at Irina I (11%) include *Methanothermococcus thermolithotrophicus* (99% sequence similarity). At Irina II, 9% of the sequences were only distantly related (94%) to *Methanocaldococcus janaschii*. Additionally at Site B a single sequence was identified as a member of the ANME-2 lineage.

Discussion

Our study was the first assessment of the microbial community inhabiting the LHF. The geological setting of the ultramafic-hosted field results in the release of high hydrogen concentrations within the emanated fluids (Table 1). Possibly, as a consequence, a large fraction of the

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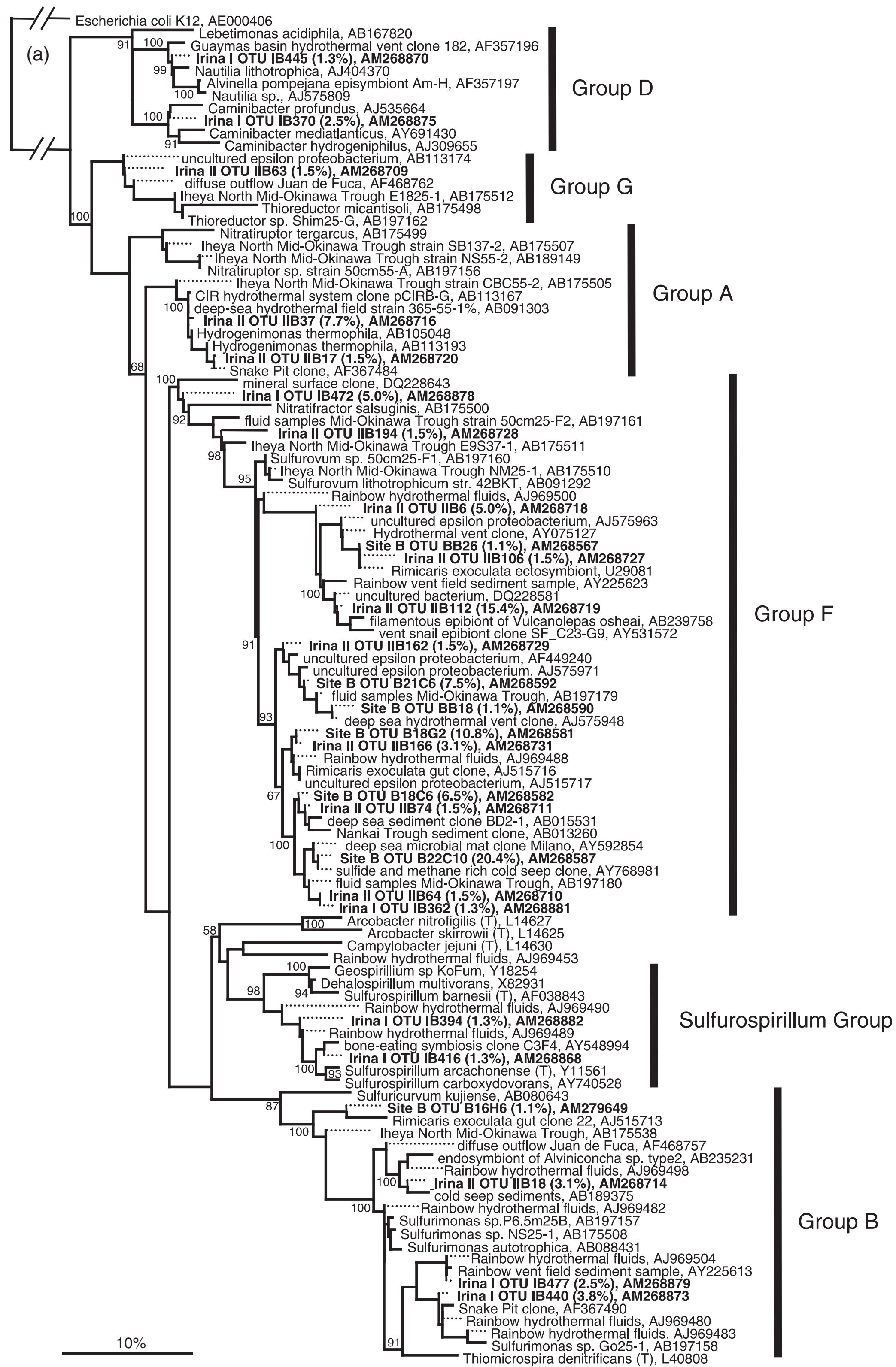

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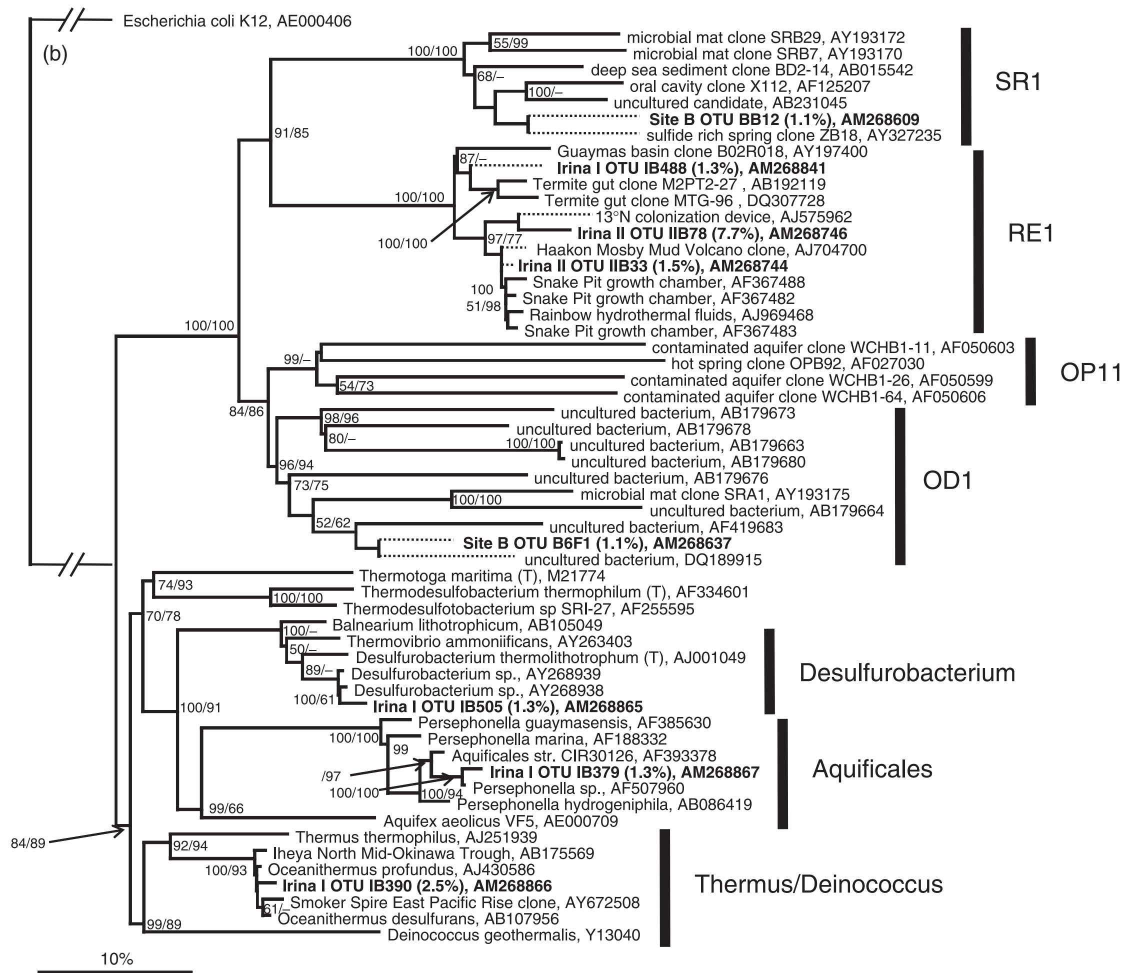

Fig. 1. Continued

microorganisms inhabiting the LHF was associated with hydrogen-oxidizers (Fig. 1a, b and d).

The archaeal community from all studied vent locations was very similar and detected archaea were mainly affiliated to *Methanococcales* (Fig. 1d). In contrast, the bacterial communities varied at the three locations studied. It seemed that an increase in hydrogen results in a diversification of potential hydrogen-oxidizing bacteria. Nonetheless, the microorganisms detected were associated with cultured prokaryotes differing in growth temperatures and tolerances towards oxygen. This strongly suggests the importance of mixing processes next to the abundance of available energy sources for the inhabitance of the hydrothermal biotopes.

The selection of ultramafic rocks for microorganisms

Methanococcales use hydrogen and CO_2 as a substrate for hydrogenotrophic methanogenesis (Whitman *et al.*, 1992). The prevalence of archaeal sequences associated with the

Fig. 1. Phylogenetic relationships of 16S rRNA gene sequences of (a) *Epsilonproteobacteria*, (b) deeply rooted bacterial lineages, (c) *Deltaproteobacteria* (*Desulfobulbaceae*) and (d) *Archaea* as determined by maximum likelihood (ML) analysis of (a) 1200, (b) 1300, (c) 1400 and (d) 1300 nucleotides. Additionally, for sequences of deeply rooted lineages (b), maximum parsimony (MP) analysis was conducted. The percentage of bootstrap resamplings above 50% is indicated. Bootstrap probabilities estimated by ML and MP analyses (b) are displayed as ML/MP. Tree topologies not supported by MP are indicated by ' – '. Dotted lines mark shorter sequences added subsequently to tree. Sequences obtained from Irina I, Irina II and Site B are listed in bold. Numbers in parenthesis indicate percentage of sequences belonging to one phylotype. The scale bar represents the expected number of changes per nucleotide position.

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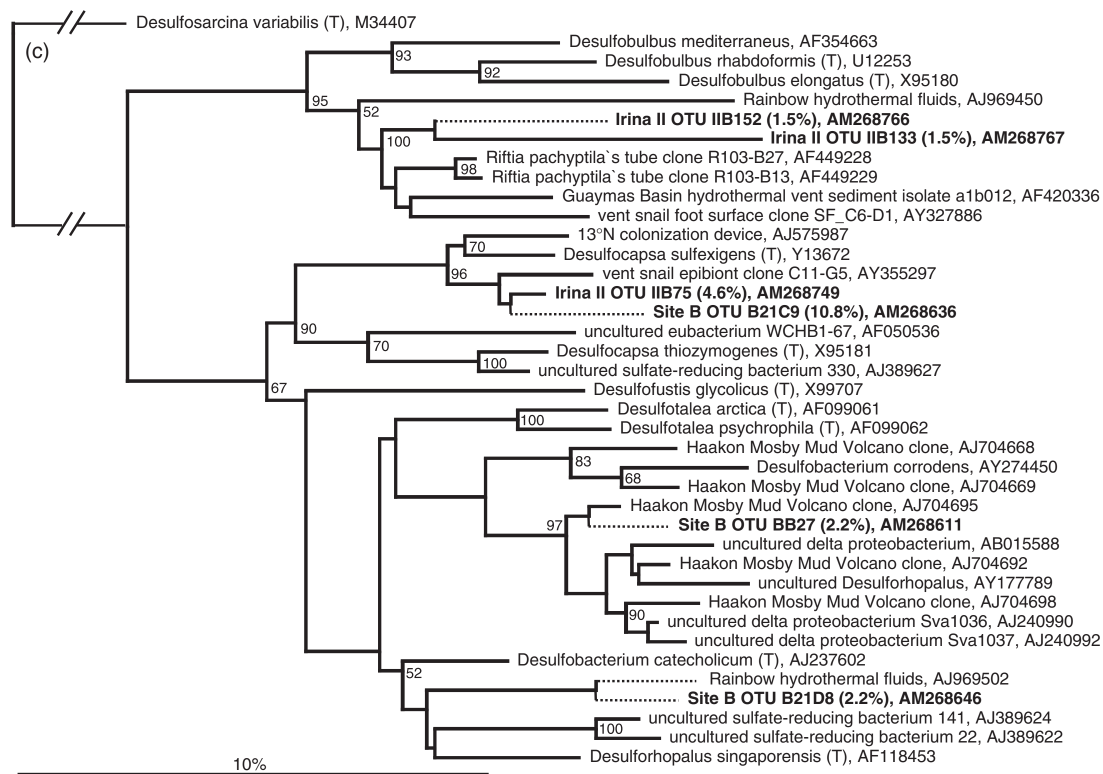

Fig. 1. Continued

Methanococcales at all investigated LHF sites (Fig. 1d) might indicate a selection for methanogenic archaea by the ultramafic-hosted system. Stable carbon isotope signatures determined for methane do not hint at a significant proportion of biogenic methane. This argues against the metabolic significance of *Methanococcales* in the LHF. Nevertheless, a biogenic methane signature might be masked due to a high background level of nonbiogenic methane.

Representatives of the *Epsilonproteobacteria* and of deeply rooted lineages have been associated with hydrogen oxidation (L'Haridon *et al*., 1998; Götz *et al*., 2002; Campbell *et al*., 2006). In contrast to the archaeal community, the fluids at each of the three vent emission sites support different bacterial communities (Fig. 1a–c). It seemed that an increase of hydrogen led to a more diverse community of putative hydrogen-oxidizing bacteria. For example, at Irina I, where the highest hydrogen concentrations were found in this study, potential hydrogen-oxidizing affiliates were distributed throughout the entire bacterial domain. These included *Aquificales* and *Epsilonproteobacteria* (group D) (Fig. 1a and b). At Irina II, where significantly lower hydrogen concentrations were found, less diverse potential hydrogen-oxidizing bacteria were present (Fig. 1a, Table 1). They were limited to the epsilon subdivision of the *Proteobacteria* (groups A and G). In contrast, at Site B, which had only slightly lower hydrogen contents than Irina II, no typical hydrogen-oxidizing bacteria were observed. The organisms detected here were affiliated to groups B and F (Fig. 1a). Only some associates of these groups have been shown to be capable of hydrogen oxidation, while others are able to utilize reduced sulfur compounds (Campbell *et al*., 2006). However, studies conducted on the NiFe-Uptake Hydrogenase (catalyzes the oxidation of molecular hydrogen), from fluids emitted at Irina II and Site B, demonstrated the potential of diverse *Epsilonproteobacteria* to be involved in hydrogen oxidation (M. Perner, unpublished data). The presence of putative hydrogen-oxidizers at all LHF locations might indicate that the ultramafic rock hosting the hydrothermal system selects for organisms with specific physiologies (hydrogen oxidation). If the nature of the host rock was the only factor influencing the distribution of the microorganisms, one would expect this to be reflected in diversity analyses obtained from other ultramafic-hosted systems. However, this is not the case (Table 1).

Out of all the active venting sites, the Rainbow vent field, with its geological setting and fluid-physico-chemistry, is the one most similar to the LHF (Donval *et al*., 1997; Lackschewitz *et al*., 2005). Nonetheless, in Rainbow hydrothermal environments hydrogen-oxidizers such as *Aquificales* or of members associated with e.g. groups A or D (*Epsilonproteobacteria*) were not detected (López-García *et al*., 2003) (Table 1). However, the three LHF locations

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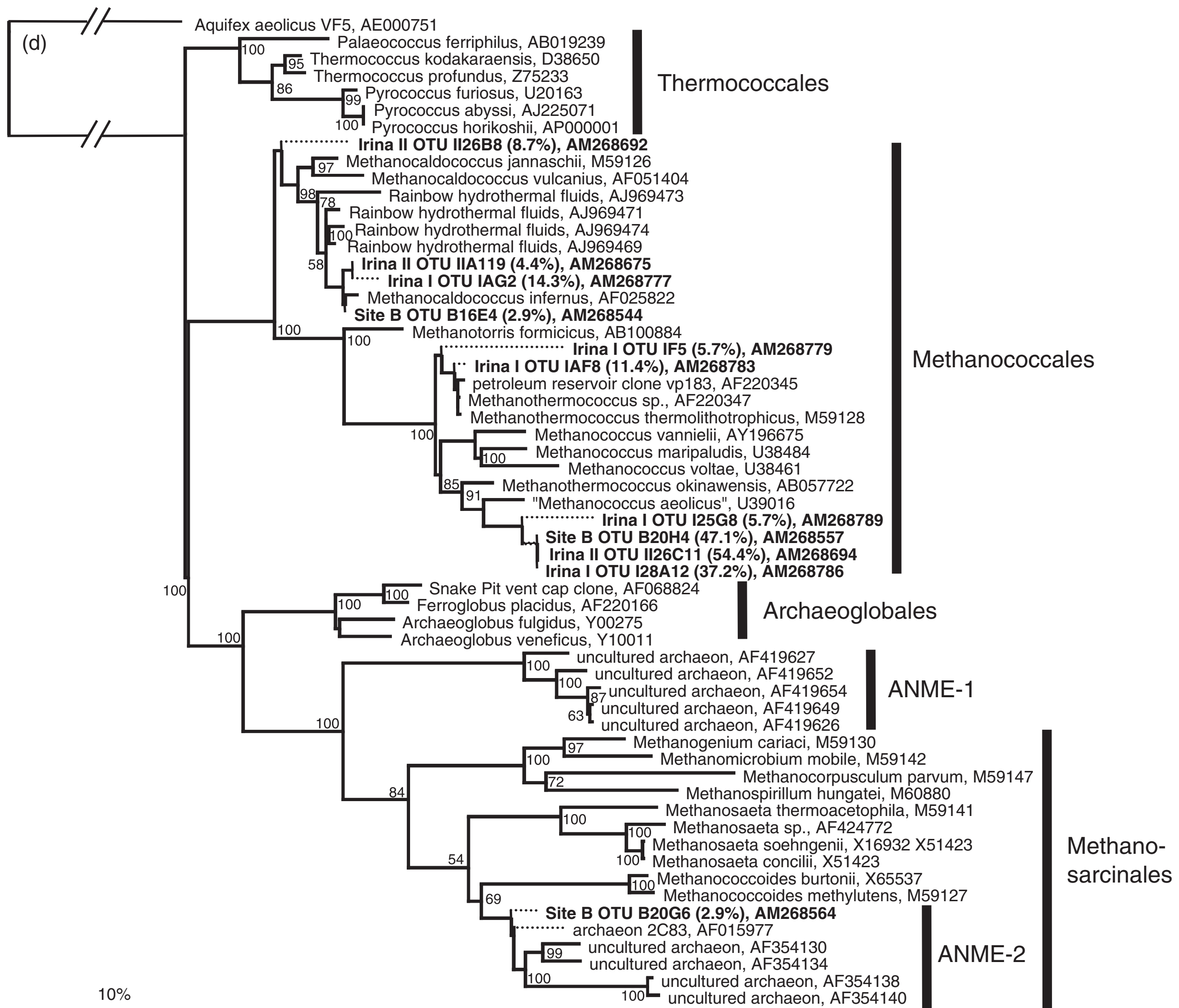

Fig. 1. Continued

have shown that bacterial diversity can differ significantly even though general chemical parameters seem to be similar (Table 1). In contrast, *Methanococcales* were found at the Rainbow field using functional gene analysis (Nercessian *et al*., 2005). This is in agreement with archaeal communities observed in the LHF emissions (Fig. 1d). Although the Lost City vent field is ultramafic-hosted and fluids have high hydrogen concentrations, the physico-chemical features of the emitting fluids are very different to those found in the LHF and the Rainbow field (for references see Table 1). The role of methane as a microbial energy source seems to be pronounced in the Lost City environment (Brazelton *et al*., 2006). Again, however, even though high hydrogen contents were ascertained, no typical hydrogen-oxidizing organisms were apparent (Brazelton *et al*., 2006). However, hydrogen oxidation has been suggested for some prokaryotes (Brazelton *et al*., 2006).

In contrast to the ultramafic-hosted systems described above, basalt-hosted hydrothermal systems typically reveal lower hydrogen contents (e.g. Van Dover *et al*., 2001; Von Damm & Lilley, 2004). Despite the low hydrogen concentrations of these basalt-hosted environments, microbial communities consisted in part of organisms related to phylogenetically diverse hydrogen-oxidizing microorganisms (e.g. Hoek *et al*., 2003; Kormas *et al*., 2006) (Table 1). As microorganisms detected at Site B show, relatively high concentrations of hydrogen do not necessarily result in phylogenetically diverse hydrogen-oxidizing communities. This set of findings indicates the importance of several other parameters essential in controlling the composition of the microbial population. The large number of phylogenetically diverse species affiliated with organisms characterized by various temperature and oxygen requirements suggests that mixing processes (hydrothermal fluids and oxygenated

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seawater) play an important role for the microbial community.

The importance of mixing processes

Our frequent encounters of bacteria and archaea related to cultured representatives with different temperature and oxygen requirements suggests mixing processes at the three vent locations. As hydrothermal fluids rise to the surface they mix with ambient seawater and cause physico-chemical gradients. These physico-chemical features are reflected in the physiologies of organisms adapted to these environments. Therefore the detection of specific groups can give an indication of the intensity of these mixing processes. The highest phylogenetic diversity throughout the bacterial domain was observed at Irina I. It included members of *Epsilonproteobacteria* and deeply rooted lineages (Fig. 1a and b). A thermophilic lifestyle is a group-specific characteristic of some of these representatives (L'Haridon *et al*., 1998; Götz *et al*., 2002; Campbell *et al*., 2006). Again, in emissions from Irina II, organisms were detected which were linked to thermophilic *Epsilonproteobacteria*. However, these *Epsilonproteobacteria* are characterized by slightly lower growth temperatures compared to cultured members of the deeply rooted lineages found at Irina I but absent from Irina II. This implies that the habitable environments at Irina I allow less intense mixing processes than the biotopes at Irina II. At Site B, no prokaryotes were observed related to thermophilic bacteria. The only microorganisms typically known for a thermophilic lifestyle included archaea of the order *Methanococcales* (Fig. 1d). *Epsilonproteobacteria* inhabiting Site B biotopes were exclusively affiliated to members of groups B and F. Their cultured representatives have been described as mesophiles (for review see Campbell *et al*., 2006). This could indicate that bacterial life at this location is only possible in environments where dilution of the hydrothermal fluids is ensured.

The presence of chemical gradients observed is also reflected in different levels of oxygen requirements characteristic for certain cultivated groups. As shown, several organisms at Irina I were related to bacteria with a thermophilic lifestyle. This suggests less intense mixing with oxygenated water. However, alongside sequences related to strictly anaerobic organisms were those also associated with aerobic and microaerophilic bacteria (Fig. 1a–c). Therefore, oxygen must be available in some areas. Nevertheless, merely one phylotype of group F *Epsilonproteobacteria* was encountered at Irina I (Fig. 1a). Judging from the group-specific characteristics of these bacteria, they are not only associated with lower growth temperatures but also with tolerance towards oxygen (Campbell *et al*., 2006). Members of this group were, however, detected in great diversity at Irina II and Site B. Their occurrence in the fluids next to anaerobic *Desulfobulbaceae* implicates mixing processes which have caused multiple biotopes to arise along the fluid pathways. The absence of *Deltaproteobacteria* at Irina I might indicate a limitation of sulfate or other oxidized sulfur species, or the influence of oxygen on the selection of bacteria.

The presence of thioautotrophic and methylotrophic symbionts of the vent mussel *Bathymodiolus* spp. in outflows at Irina II is not surprising, as *Bathymodiolus* assemblages colonize the entire surroundings (Kuhn *et al*., 2004; Lackschewitz *et al*., 2005). Predation or natural death of *Bathymodiolus* spp. could explain the occurrence of symbionts among free-living prokaryotes of the area.

Because thermophilic *Methanococcales* have been detected in low-temperature emissions, it has been argued that they might originate from subsurface environments (Huber *et al*., 2002; Takai *et al*., 2004a; Nakagawa *et al*., 2005a). They are independent of seawater-derived oxidants. The uniformity of the archaeal community at the LHF could suggest that parts of the *Methanococcales* originate from the subsurface.

Other environmental parameters

A single sequence at Irina I OTU IB488 and 10% of bacterial sequences from Irina II fluids were affiliated with the new group (RE1) (Fig. 1b), of which no cultured representatives exist. As all sequences of this group are derived from reduced environments, the name of RE1 for 'reduced environment' is proposed. It is conspicuous that, with the exception of two sequences retrieved from termites, all others originate from hydrothermal vent environments (Fig. 1b). The chemical conditions, i.e. low sulfide concentrations and high hydrogen concentrations (Table 1), could be favorable for the occurrence of RE1 members. However, several sequences of this group are derived from Snake Pit (Corre *et al*., 2001). Significantly higher sulfide concentrations (6 mM) have been determined at Snake Pit (Douville *et al*., 2002) than at the LHF (Table 1). The role these uncultured affiliates play in the ecosystem remains to be investigated.

For the first time, we report a sequence affiliated with the ANME-2 lineage of the *Methanosarcinales* from hot hydrothermal emissions. Interestingly, it was detected at Site B, where the lowest methane concentrations were measured (0.6 mM) (Table 1, Fig. 1d). Affiliates of ANME-2 mediate the anaerobic oxidation of methane and have been found in anoxic sediments, seep environments and also hydrothermally active sediment (e.g. Boetius *et al*., 2000; Orphan *et al*., 2001). The question of whether the sequence affiliated with the ANME-2 group is associated with methane oxidation remains open, as no significant methane oxidation rates were measured at diffusive and hot emission sites at the LHF (J. Felden, pers. commun.).

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