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Molecular ecology of hydrothermal vent microbial communities

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Abstract

The study of the structure and diversity of hydrothermal vent microbial communities has long been restricted to the morphological description of microorganisms and the use of enrichment culture-based techniques. Until recently the identification of the culturable fraction required the isolation of pure cultures followed by testing for multiple physiological and biochemical traits. However, peculiar inhabitants of the hydrothermal ecosystem such as the invertebrate endosymbionts and the dense microbial mat filaments have eluded laboratory cultivation. Substantial progress has been achieved in recent years in techniques for the identification of microorganisms in natural environments. Application of molecular approaches has revealed the existence of unique and previously unrecognized microorganisms. These have provided fresh insight into the ecology, diversity and evolution of mesophilic and thermophilic microbial communities from the deep-sea hydrothermal ecosystem. This review reports the main discoveries made through the introduction of these powerful techniques in the study of deep-sea hydrothermal vent microbiology.

Introduction

The discovery of deep-sea hydrothermal vents along the East Pacific Rise (EPR) has significantly modified the established views on the deep sea that was long known as a cold, dark, high-pressure and nutrient-poor environment (Corliss et al. 1979). The unexpected presence of luxuriant populations of organisms clustering around emissions of geothermally generated reduced compounds challenged the notion that life in the deep-sea is limited by reduced carbon and energy. In this spatially-limited, productive ecosystem partially insulated from solar radiations, it soon became apparent that the source of organic carbon in the vent animals originated from the interaction of chemosynthetic micro-organisms with the emitted, reduced, chemical species (Rau & Hedges 1979; Rau 1981; Jannasch 1995). Ensuing years have seen the discovery and intensive study of other hydrothermal vent environments in both the Atlantic and Pacific oceans.

The first investigations of hydrothermal vent microbial communities employed numerous methods to determine microbial diversity, biomass, physiological potential, and production rates of the main microbial assemblages. At least four generic hydrothermal vent habitats and associated microbial communities are now known to exist; they include (i) free-living bacterial populations associated with the discharged vent fluids and presumably growing and reproducing within the sub-seabed system, (ii) free-living microbial mats growing on surface strata that are exposed to flowing vent waters, (iii) endo- and exosymbiotic associations of microorganisms and vent fauna, and (iv) microorganisms within the deep sea hydrothermal vent plumes (Karl 1995).

Studies of the microbial diversity associated with deep-sea hydrothermal vents have for a long time been restricted to the microscopic description of samples and the isolation of a restricted number of new mesophilic microorganisms. Considering the multiple ecological niches suitable for their life across the vent habitats (cited above), mesophiles may represent the most abundant bacteria in hydrothermal vents. However, very little is known about their taxonomic and ecological significance (Baross & Deming 1995). Due to the recent fascination of microbiologists with ther-

mophiles, a greater number of thermophilic isolates has been obtained from hydrothermal vents (Stetter 1996a). Therefore, much more is known about thermophiles presumed to inhabit thermally restricted environments. However, it is now generally accepted that the vast majority of microorganisms detectable by direct methods in marine and other natural samples, including those collected from much less extreme environments, cannot be assessed by traditional methods that rely on laboratory cultivation (Ward et al. 1992). Consequently, results derived from elective culture studies may be of limited use in microbial ecology. In addition, substantial difficulties met in recovering representative samples, developing appropriate media, and recreating the extreme conditions of deep-sea hydrothermal environments in the laboratory have prevented microbiologists from gaining access to the extent and nature of the existing microbial diversity.

These technical hurdles have been partly overcome by the development of molecular biological techniques, especially those that take advantage of rRNA molecules and their encoding genes. These were perceived to have a number of characteristics that made them eminently suitable for phylogenetic and determinative studies (Woese 1987). The rRNAs are essential components of the protein synthesis machinery and are therefore widely distributed and functionally conserved in all organisms. They are ancient molecules and are extremely conserved in overall structure, making them readily isolatable and identifiable. Their primary structures are composed of sequence regions of higher and lower evolutionary conservation. They lack the interspecies horizontal gene transfer found in many prokaryotic genes. The 5S and 16S rRNAs have been used most frequently for rRNA-based phylogenetic characterizations. However, the paucity of independently varying nucleotide positions in the 5S rRNA molecule has limited its phylogenetic usefulness (Olsen et al. 1986). The larger and therefore more informative 16S rRNA molecule is of an appropriate size for broad phylogenetic analyses. Moreover, 16S rRNA sequences have already been determined for a large fraction of the validly described species and their natural amplification with high copy numbers per cell greatly increases the sensitivity of rRNA-targeted probing.

This review reports on the use of molecular methods such as DNA-DNA hybridization (Sayler & Layton 1990) and lipid analysis (Tornabene 1985) but will mainly concentrate on the application of the approaches involving the analysis of rRNA sequences in organisms found in the hydrothermal environment (Olsen et al. 1986; Pace et al. 1986; Stahl & Amann 1991; Ward et al. 1992; Amann et al. 1995). For instance, specific 16S rRNA-targeted oligonucleotide probes have been successfully introduced as powerful tools in microbial vent ecology (Table 1). They have been applied to the localization of invertebrate-associated endo- and ectosymbionts, to the quantification of a yet uncultivable dominant phylotype in low-temperature communities, and to the distribution of thermophilic microorganisms in vent chimneys. These three genetically diverse natural assemblages of deep-sea hydrothermal vent environments will be discussed successively.

Molecular studies of invertebrate-associated microbial populations

Epibionts of Alvinella pompejana

Alvinella pompejana is a tube-dwelling annelid polychaete endemic to the EPR; it inhabits the hottest areas of active deep-sea hydrothermal vent chimneys. The harsh chimney environment is characterized by extreme temperature gradients (2 to 350 °C) and high concentrations of hydrogen sulfide (>1 mM) and toxic heavy metals. Heavy metal concentrations measured in undiluted hydrothermal fluids appear consistently higher than in the surrounding non-hydrothermal deep-sea water (Michard et al. 1984; Von Damm et al. 1985a,b; Bowers et al. 1988). Zinc and copper are among the most abundant and form a large part of the polymetallic sulfide edifices. In this environment of sharp physico-chemical gradients with tremendous temporal and spatial variability, reliable recording of conditions within the worm's immediate microenvironment is difficult. Temperature ranges of 25–40 °C have been recorded at the opening of the worm tubes. Temperatures of 40–50 °C or even 70–80 °C have been measured inside the tube masses and an individual of the genus *Alvinella* was reported to have survived temperatures in excess of 100 °C for a prolonged period of time (Chevaldonné et al. 1992). Data obtained from physiological and biochemical studies on isolated organelles or molecules (reviewed in Childress & Fisher 1992) indicate that *A. pompejana* must be limited to temperatures lower than 50 °C. These authors suggested a probable optimal body temperature of 30–35 °C. However, in six independent surveys, temperature recorded 6 to 8 cm within the worm tube

Table 1. Sequences, target sites, and specificities of rRNA-targeted oligonucleotide probes for whole-cell hybridizations useful for the identification of microorganisms known to thrive in the deep-sea hydrothermal vent environment. Some of these probes have been designed to identify the same organisms, populations or communities in other habitats

Sequence (5′–3′) of probe	Target position	Specificity	Reference
Universal			
ACGGGCGGTGTGTRC	1392–1406	Universal	Lane et al. (1985)
GWATTACCGCGGCKGCTG	522–536	Universal	Giovannoni et al. (1988)
Domain level			
GCTGCCTCCCGTAGGAGT	338–355	*Bacteria*	Amann et al. (1990)
ACCGCTTGTGCGGGCCC	927–942	*Bacteria*	Giovannoni et al. (1988)
GTGCTCCCCCGCCAATTCCT	915–934	*Archaea*	Amann et al. (1990)
Upper group level			
CCAGRCTTGCCCCCCGCT	449–515	*Crenarchaeota*	Burggraf et al. (1994)
CTTGCCCRGCCCTT	498–510	*Euryarchaeota*	Burggraf et al. (1994)
GTTTGCCCGGCCAGCCGTAA	1116–1135	*Korarchaeota*	Burggraf et al. (1997)
Lower group level/genus level			
GGGTTTCGTCCCGGGTTC	438–455	*Thermus aquaticus*, *T. flavus*, *T. thermophilus*	Harmsen et al. (1997b)
GGGTGTGACCCCTCTAAC	832–849	Thermophilic *Bacillus* spp.	Harmsen et al. (1997b)
GTTCCGTCTCCCTCTACC	660–677	*Thermosipho* spp. and *Thermotoga* spp.	Harmsen et al. (1997b)
TCGCGCAACGCTCGGGACC	540–558	*Aquificales*	Harmsen et al. (1997b)
GCAACATAGGGCACGGGTCT	1109–1128	*Methanococcus*[a]	Raskin et al. (1994)
GCGATTACTACG(G/C)ATTCCAG	1335–1354	All methanogens[b] and *Archaeoglobus*	Torsvik et al. (1993)
Species level			
TCCCTGTTGCCAGGTCGC	1258–1275	*Thermus aquaticus*	Byers et al. (1997)
TCCCTGTCACCAGGTCGC	1258–1275	*Thermus filiformis*	Byers et al. (1997)
TCCCCGTTACCGGGTCGC	1258–1275	*Thermus ruber*	Byers et al. (1997)
TCCCCGTTGCCGGGTGGC	1258–1275	*Thermus thermophilus*	Byers et al. (1997)
GTTTGCAGCCCTCTGTACCGG	1243–1263	*Riftia pachyptila* symbiont	Cary et al. (1993)
ACTACCTCACGGCTRAGCGAC	1255–1275	*Calyptogena magnifica* symbiont	Cary et al. (1993)
TTGCTCTCGCGAGGTTGCGAC	1255–1275	*Bathymodiolus thermophilus* symbiont	Cary et al. (1993)
ATCTTCCCCTCCCAGACTCT	653–672	*Rimicaris exoculata* symbiont	Polz & Cavanaugh (1995)
Environmental clones			
GTCCCCTGCCTCCCTTGC		Environmental clone EM17 (*Aquificales*)	Reysenbach et al. (1994)
TGCCTTCACCCCACCTTC		Environmental clone EM3 (*Thermotogales*)	Reysenbach et al. (1994)
CACACATTCGTCCCTTTT		Environmental clone EM19	Reysenbach et al. (1994)
AGTTTTGCTTCTCTTTGTCCTT	1244–1265	ε proteobacterial phylotype 13B	Cary et al. (1997)
GATTTCGCTTCTCATTGTCCTC	1244–1265	ε proteobacterial phylotype 5B	Cary et al. (1997)
GTCGCCAGCACTATTACC	89–106	Environmental clones (*Aquificales*)	Yamamoto et al. (1998)

[a] One mismatch with *M. jannaschii*.
[b] Except *Methanopyrus kandleri*.

averaged 68±6.33 °C over a 3-h period with frequent spikes exceeding 81 °C and a temperature gradient of up to 60 °C was measured along the length of the worm's body, suggesting that *A. pompejana* is the most eurythermal and thermotolerant metazoan known (Cary et al. 1998).

One of the most striking features of this worm is its obligate association with a highly diverse and dense assemblage of epibiotic microorganisms. Dorsal epidermal expansions are externally covered by filamentous morphotypes that dominate the worm-bacteria association. Rod-shaped, prosthecate, spiral-curved, filamentous sheathed or unsheathed bacteria are scattered on the surface of the worm integument whereas clump-like assemblages of rod-shaped, coccoid and filamentous bacteria are associated with cuticular protrusions in the intersegmental spaces [Gaill et al. 1987; see Desbruyères et al. (1998) for review]. Metabolically diverse non-filamentous mesophilic bacteria were successfully cultured from *A. pompejana*'s dorsal integument (Prieur & Jeanthon 1987; Prieur et al. 1990; Raguénès et al. 1996, 1997a, b).

Most of the heterotrophic strains isolated from alvinellid polychaetes and their tubes displayed resistance to arsenate, cadmium, silver, and zinc and tolerated high amounts of copper (Jeanthon & Prieur 1990). The occurrence of multiple heavy-metal resistant phenotypes within these isolates, presumptively identified as members of the genera *Acinetobacter*, *Alteromonas*, *Pseudomonas*, and *Vibrio*, demonstrated an adaptation of a part of the alvinellid-associated microflora to the general enrichment of metals in the hydrothermal vent environment. Almost 20% of the isolated bacteria harboured one or more plasmids (up to five) of sizes ranging from 4.6 to 157 kb (Jeanthon 1991; Jeanthon et al. 1991). Among the 4 to 5 different plasmid bands found in 19 *Acinetobacter* strains, three had identical sizes (4.8, 9.4 and 45.3 kb). Further hybridization experiments indicated that homologies were shared by the 45.3-kb plasmids. A 110-kb plasmid was also found to occur in *Pseudomonas* and *Vibrio* strains whereas phenotypically different strains of the genus *Acinetobacter* carried a 51.7-kb plasmid. These data indicate that transfer of genetic elements (i.e. broad host range plasmids) might take place in deep-sea environments.

To date, the dominant filamentous morphotypes have eluded all attempts at culturing. By constructing a clone library of 139 16S rDNA genes amplified by PCR from genomic DNA extracted from bacteria collected from the dorsal surface of an *A. pompejana* specimen, Haddad et al. (1995) characterized the dominant members of the associated microflora. Among the thirty-two clone families identified in the library by their unique restriction patterns, only four represented 65% of the clones. Phylogenetic analysis of the 16S rRNA genes of the four dominant clone families placed them in two distinct clades within the ε subdivision of the *Proteobacteria* (also referred as the *Campylobacter-Thiovulum* subdivision). The ϵ subdivision of the class *Proteobacteria* encompasses the genera *Arcobacter*, *Campylobacter*, *Helicobacter*, *Sulfurospirillum*, *Thiovulum* and *Wolinella* (On et al. 1998). The majority of these genera are known to be adapted to environments low in oxygen and are involved in multiple metabolic pathways regarding oxidation and/or reduction of sulfur compounds in microaerophilic and/or anaerobic conditions (Wirsen & Jannasch 1978; Vandamme et al. 1991). Other evidence for the presence in low-temperature, sulfide-dependent niches, of organisms related to the ε subdivision of the class *Proteobacteria* (see the next sections) argue for their important contribution in the sulfur cycle and ecology in hydrothermal vent environments.

Two of the phylotypes (defined as organisms with identical 16S rRNA genes), designated 13B and 5A, that dominated the clone library were identified from six different worm specimens collected from geographically separated sites, indicating that they are constant features of the associated microflora. They were found to be localized on *A. pompejana* by using *in situ* hybridization demonstrating that they are the predominant filamentous bacteria on the dorsal integument of the worm (Cary et al. 1997). The occurrence of phylotype 13B on the exterior surfaces of other invertebrate genera (*Paralvinella* sp., *Riftia pachyptila* tube) and rock surfaces and the detection of phylotype 5A on *A. caudata* integument indicated that these ε proteobacterial phylotypes do not have an obligate requirement for *A. pompejana*. However, the metabolic potential of these dominant and constant phylotypes and possible function of their relationship with the worm remain to be fully demonstrated.

Epibionts of Rimicaris exoculata

The highly motile alvinocaridid shrimp *Rimicaris exoculata* is endemic in the Mid-Atlantic Ridge (MAR). *R. exoculata* is the dominant invertebrate at least at two hydrothermal sites (Snake Pit, 23° N, and

TAG, 26° N) and clusters on solid surfaces around warm vent emissions (15–40 °C). Although phylogenetically and geographically distant, *A. pompejana* and *R. exoculata* exhibit anatomical, functional and behavioural analogies which relate to their epibiosis. The digestive tract of both species is functional and the stomach contents primarily comprise organic matter and polymetallic sulphide particles associated with the chimney walls.

One of the most unusual features of the shrimps is the extremely dense bacterial population that grows on setae attached to their hypertrophied mouthparts (Van Dover et al. 1988). Morphologically different rods and filaments of various thicknesses have led several authors to claim the presence of two or three distinct ectobacterial species (Van Dover et al. 1988; Gebruk et al. 1993).

Since the shrimp bacteria could not be cultivated, the 16S rRNA phylogenetic framework was used to characterize the epibionts (Polz & Cavanaugh 1995). A single phylotype was recovered from nucleic acids extracted from the shrimp-associated microorganisms. It forms a deep branch in the ε subdivision of the class *Proteobacteria* and a moderate relationship exists between the *R. exoculata* symbiont and *Thiovulum*, a sulfide-dependent marine bacterium. An epibiont-specific 16S rRNA-targeted oligonucleotide probe was designed to test if this dominance was reflected in the epibiont population *in situ*. *In situ* hybridization and epifluorescence microscopy confirmed the genetic identity of all three different morphotypes of the shrimp epibionts at the 16S rRNA level and suggested that a single species (maybe pleomorphic) or multiple species with identical 16S rRNA grow on the shrimp body. Most surprisingly, the epibiont phylotype was not restricted to the shrimp body and was found to contribute over 60% of the detectable rRNA on the surfaces of solid sulfide blocks from the same hydrothermal site whereas a complete absence of signals from the probes specific to the domains *Archaea* and *Eukarya* was observed.

The *R. exoculata*-associated phylotype may benefit from the animal's movement to get a continuous access to a stable environment rich in reduced sulfur compounds in the gradients formed in the mixing zone of reduced and oxidized waters but the exact role of epibiotic symbionts for their host is not clear yet. However, the results obtained by using a combination of different molecular techniques suggest that the shrimp is involved in a nutritional symbiosis with their epibionts (Polz et al. 1998).

Chemoautotrophic endosymbioses

Other invertebrates like the vestimentiferan tube worm *Riftia pachyptila*, the vesicomyid clam *Calyptogena magnifica*, and the mytilid bivalve *Bathymodiolus thermophilus* dwell in environments surrounding hydrothermal vents and are characterized by the maintenance of chemoautotrophic sulfur-oxidizing endosymbiotic bacteria in specialized tissues of their body (see for review Nelson & Fisher (1995)). Despite the characterization of the symbionts being hampered by their resistance to cultivation apart from their hosts, the evolutionary origin of the chemoautotrophic endosymbionts was elucidated by 5S and 16S rRNA comparative sequence analyses (Stahl et al. 1984; Distel et al. 1988, 1994). The sequence analyses were entirely consistent with the view that each host contains a single endosymbiont population.

In phylogenetic trees based on the 16S rRNA sequences, the bacteria form two deeply divergent but loosely associated groups which cluster within the γ subdivision of the class *Proteobacteria* that also encompasses free-living sulfur-oxidizing bacteria. Edwards & Nelson (1984) used the DNA–DNA hydridization technique to reveal the presence of a single symbiotic bacterial species in 13 individuals of *R. pachyptila* collected from geographically distinct vent sites. The symbiont of the distantly related vestimentiferan *Tevnia jerichonana* was found to be the same as that of *R. pachyptila* (Edwards & Nelson 1984).

Three oligonucleotide probes specific to each of the symbionts of *C. magnifica*, *B. thermophilus* and *Riftia pachyptila* were used as primers for the sensitive detection of symbiont signal (<1 pg) from bulk gonadal DNA extracts of their respective hosts and for *in situ* hybridization experiments to localize the position of symbiont signal in thin sections of host tissues (Cary et al. 1993). PCR amplification with DNA extracted from eggs of *R. pachyptila* was not successful. The probe specific to the *R. pachyptila* symbiont was also found to be effective for the symbiont of *Ridgea piscesae*; *in situ* hybridization studies did not detect symbiont target in mature and immature oocytes of this vestimentiferan. These findings suggest that the bacteria must be acquired after settlement.

The above methods and approach were successfully used to indicate the presence of specific symbiont 16S rDNAs in the ovary of three species of the genus *Calyptogena* and to localize the symbionts to follicle cells surrounding the developing oocytes. These results provided strong evidence for a vertical transmis-

sion of the symbionts in these vesicomyid clams, a mechanism of inheritance also revealed in the mytilid *B. thermophilus* (Cary & Giovannoni 1993).

As has been widely reported for thioautotrophic associations, monospecific endosymbioses with methanotrophic bacteria are also widespread among mytilids (Nelson & Fisher 1995). The stable coexistence of both types of symbiotic bacteria in a multicellular eukaryote (e.g. in some yet undescribed mussels from the Snake Pit vent site, at a depth of 3476 m on the MAR) has also been demonstrated (Cavanaugh et al. 1992; Distel et al. 1995). Both symbionts are closely related to the thioautotrophic and methanotrophic symbionts previously found in monospecific associations in deep-sea hydrothermal vents and hydrocarbon seeps (Distel et al. 1995). *In situ* hybridization experiments confirmed that they were both contained in a single gill bacteriocyte and showed that the thioautotrophic symbiont type was dominant in mussel specimens from this vent site (Distel et al. 1995). Stable carbon isotope ($\delta^{13}C$) ratios of the mytilid tissues, typical of thioautotroph-bivalve symbioses, were also consistent with a presumed higher contribution of the thioautotrophic symbionts (Cavanaugh et al. 1992). Recently, the composition of fatty acids diagnostic for thio- and methanotrophic bacteria confirmed the presence of both bacterial endosymbiont types in the gill tissues of an undescribed *Bathymodiolus* sp. that is dominant at the Menez Gwen vent site (850 m depth on the MAR) (Pond et al. 1998). The fatty acid composition in host tissues with and without the symbionts indicated that chemoautotrophy makes a contribution to tissue carbon.

The data from the stable carbon isotope composition analysis suggested that both endosymbionts were equally important for the nutrition of the mussel host. At this particular vent site, emissions are richer in methane than is the case at deeper sites of the MAR where thiotrophic bacteria-shrimp symbioses dominate (i.e., Snake Pit vent site). Thus, despite the presence of a reduced but functional gut in this mussel that could potentially utilize an exogenous source of particulate food, physiologically distinct bacterial symbionts may provide a nutritional flexibility to their host and may facilitate their colonization of more than one type of chemical environment.

Molecular ecology of microbial communities from low-temperature habitats

Culture-based enrichment techniques have been extensively used to isolate members of mesophilic communities colonizing rock, chimney, sediment or animal surfaces exposed to vent emissions. The availability of dissolved free oxygen in deep-sea waters renders aerobic sulfide, sulfur and thiosulfate oxidation the predominant source of energy for the reduction of CO_2 to organic carbon (Jannasch 1995). Consistent with this has been the isolation of a variety of sulfur-oxidizing bacteria associated with the vent environment. With the exception of endosymbiotic chemolithotrophs, sulfur-oxidizers identified from the hydrothermal vent environment include: (i) yet uncultivated large filaments attributed to *Beggiatoa* and *Thiothrix* that form dense bacterial mats (Jannasch et al. 1989; Nelson 1989), (ii) numerous base-producing facultative chemolithotrophs and heterotrophs (Ruby et al. 1981; Durand et al. 1994), and (iii) rather few acid-producing obligately chemolithotrophic bacteria limited to two species, *Thiobacillus hydrothermalis* (Durand et al. 1993) and *Thiomicrospira crunogena* (Ruby & Jannasch 1982; Jannasch et al. 1985; Wirsen et al. 1998).

Data accumulated using enrichment and isolation culture techniques support the hypothesis of the dominance of mixotrophic sulfur-oxidizing populations at deep-sea vents. However, the failure to maintain *Thiomicrospira* isolates in subcultures (Durand et al. 1994) reflects the well known fastidious nature of the obligate chemolithotrophs and may lead to their underestimation in this habitat. Thus, by using a culture-independent method (Denaturing Gel Gradient Electrophoresis – DGGE analysis), species of the genus *Thiomicrospira* belonging to the γ-subdivision of the class *Proteobacteria* were found to correspond to the dominant bacterial populations in communities growing on two independent chimney rock samples (Muyzer et al. 1995). The dominant molecular isolate, identified as *T. crunogena* or a very closely related organism of identical partial 16S rRNA sequence, was associated with a second phylotype probably representing a new *Thiomicrospira* species and to a third, very closely related to the sulfate-reducer *Desulfovibrio salexigens*. The potentially dominant occurrence of sulfate-reducing bacteria in the hydrothermal vent community is consistent with the evidence for sulfate reduction activity in sediments at Guaymas Basin (Jorgensen et al. 1992; Elsgaard et al. 1994) and their

repeated isolation from various samples collected at different hydrothermal sites (Elsgaard et al. 1991, 1995).

Direct measures of microbial taxonomic diversity in low-temperature hydrothermal vent environments have been performed by analyzing the composition of the different sequence types in PCR-derived rDNA gene clone libraries. Due to the extent of biases in PCR amplification from mixtures of 16S rDNAs (Farrely et al. 1995; Suzuki & Giovannoni 1996; Chandler et al. 1997; Polz & Cavanaugh 1998), the frequency of specific sequence types amplified from natural communities from a given habitat cannot be considered representative of the relative abundance of the particular organisms in the environment. Despite the quantitative interpretation of PCR-based results being viewed with caution, the following studies demonstrated other aspects of the effectiveness of PCR-based techniques in evaluating the diversity of vent microbial communities.

Twelve distinct phylotypes were recovered in a library of 48 16S rRNA gene clones constructed from a bacterial mat collected at an active deep-sea vent ecosystem located at Pele's vents (980 m depth on the Loihi Seamount, Hawaii) (Moyer et al. 1994). Two taxa comprised 87.6% of the total 16S rDNA clonal diversity (Moyer et al. 1995). The major one, accounting for 60.5% of the clone library, was closely affiliated with a *Thiovulum* sp. within the ε-subdivision of the class *Proteobacteria*. This finding paralleled results obtained by quantitative slot-blot analysis on surface sulfides (Polz & Cavanaugh 1995). The second cluster contained within the γ subclass of the *Proteobacteria* was affiliated with a *Xanthomonas* sp. and was closely related to several obligate chemolithotrophic *Thiobacillus* spp. The close relationship of one additional phylotype with the *Alteromonas* group was consistent with the isolation of numerous strains of this genus from deep-sea hydrothermal vents (Jeanthon & Prieur 1990; Vincent et al. 1994; Raguénès et al. 1996, 1997a).

The number of archaeal phylotypes recovered from the same microbial mats was significantly greater than that revealed within the bacterial domain (Moyer et al. 1998). Four phylotypes selected after restriction fragment length polymorphism (RFLP) from a 75 clone library clustered within two distinct lineages of as a yet uncultivated marine planktonic *Archaea* (crenarchaeal group I and euryarchaeal group II) recovered from coastal subsurface marine habitats from the world's oceans (DeLong 1992; Fuhrman et al. 1992; DeLong et al. 1994).

The discovery of archaeal phylotypes in cold and temperate, oxygenated sea water was the beginning of the emerging picture showing that members of the *Archaea* inhabit a wide variety of niches and are ecologically much more important than previously thought. Prior to this, cultured *Archaea* were known to thrive only in unusual or extreme aquatic and terrestrial environments and consequently were considered to account for a limited fraction of the global biodiversity. The recent findings of relatives of the marine crenarchaeal group in association with marine invertebrates (McInerney et al. 1995; Preston et al. 1996), in continental shelf sediments (Vetriani et al. 1998), in freshwater sediments (Hershberger et al. 1996; MacGregor et al. 1997; Schleper et al. 1997) and in terrestrial soils (Ueda et al. 1995; Bintrim et al. 1997; Jurgens et al. 1997) and of relatives of the marine euryarchaeal group in coastal marsh and continental shelf sediments (Munson et al. 1997, Vetriani et al. 1998) and in winery by-products (Godon et al. 1997) greatly extends our earlier view of the phylogenetic and probably also physiological diversity of *Archaea*.

The planktonic *Archaea* cannot be vagrant thermophiles swept from their natural habitats with venting hydrothermal fluids and plumes because (i) they are widespread, abundant, and thrive in diverse environments, (ii) their 16S rDNA G+C ratios are relatively low when compared to that of known thermophiles, and (iii) the culturable hyperthermophilic *Archaea* would occur with them. However, the affiliation of the archaeal phylotypes from Pele's vents to marine planktonic lineages may strongly support a link between marine hydrothermal and pelagic habitats (Moyer et al. 1998). These authors suggested either that low-temperature hydrothermal vent habitats could act as a potential source of marine archaeoplankton or that the cold seawater circulation through the hydrothermal vent systems could concentrate these organisms in microbial mats that were exposed to the hydrothermal flow regime.

The analysis of bacterial and archaeal diversity from samples collected from deeper hydrothermal vents (Snake Pit site, MAR) shared strong similarities with that analysed in Pele's vents. To study the communities that establish over time at deep-sea vents, an *in situ* titanium growth chamber (vent cap) was designed and deployed over vent emissions. Hydrothermal fluids pass freely through the chamber which contains surfaces onto which microorganisms can at-

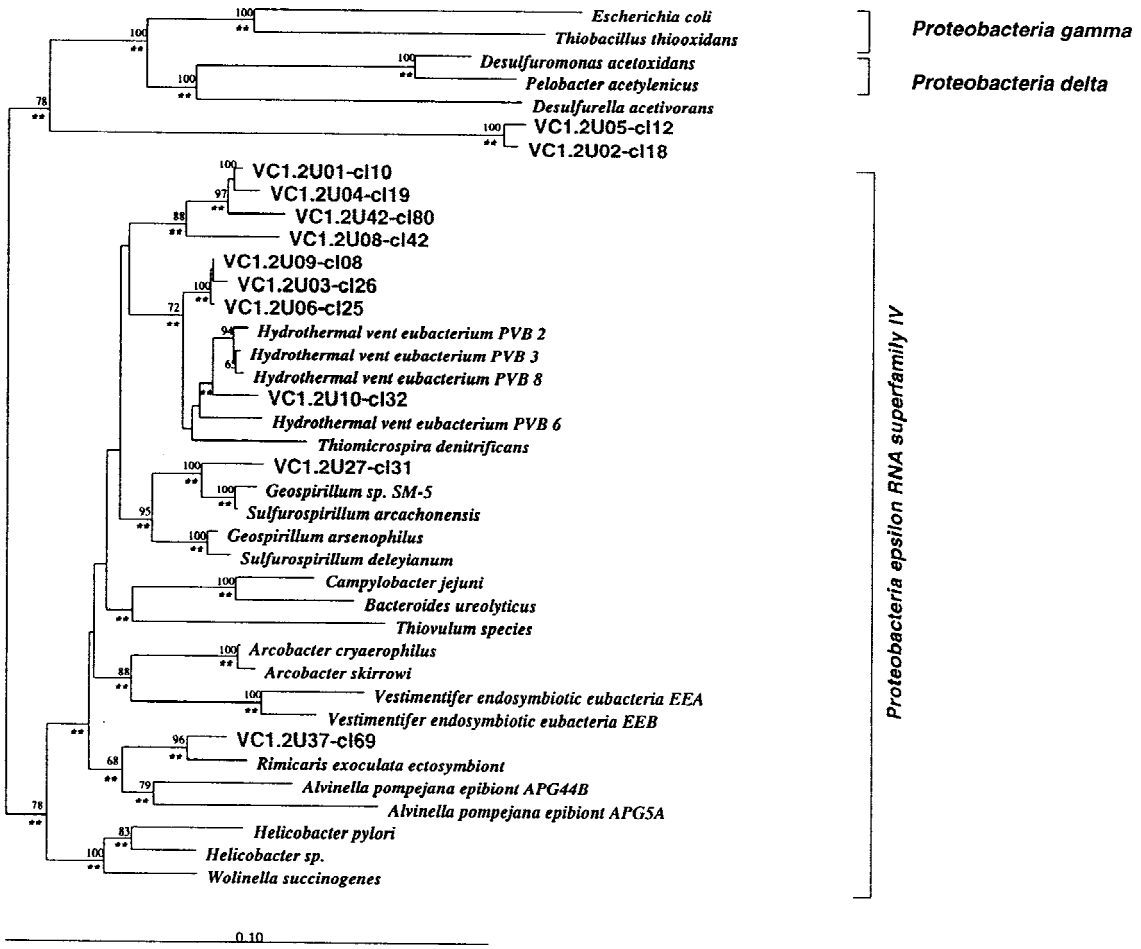

Figure 1. Phylogenetic tree demonstrating relationships of 16S rRNA sequences recovered from a vent cap (VC) sample deposited on warm hydrothermal emissions at the Snake Pit site within the *ε-Proteobacteria* as determined by neighbour-joining analysis. The 16S rDNA sequences were obtained from a 5-day deployment of a vent cap at 23° N (the average temperature recorded inside the *in situ* sampler was approximately 20 °C). Other sequences (italics) were provided by the Ribosomal Database Project (Maidak et al. 1997). Support from a bootstrap analysis using parsimony is shown above each branch as a percentage of replications (only bootstrap values >50% are indicated). Branches significantly positive at $P < 0.01$ with the maximum-likelihood method are labelled with double asterisks. The scale bar represents the expected number of changes per sequence position. With the permission of Erwan Corre.

tach. After a 5-day exposure of a vent cap on warm hydrothermal vent emissions, the bacterial community established at an average temperature of 20 °C was highly diverse (47 phylotypes determined by RFLP in a 87 clone library) and consisted exclusively of members of the ε-subclass of the class *Proteobacteria* that were most closely related with the sequences retrieved from the Pele's vent samples (Figure 1) (Corre et al. 1997).

Reysenbach et al. (1997) also reported that the *ε-Proteobacteria* were present in samples from a 2- and a 5-day deployment on other vent emissions at the Snake Pit site. However, close relatives of eubacterial and archaeal thermophiles were also present in these samples. The archaeal thermophilic lineages were related to the orders *Archaeoglobales*, *Methanococcales*, and *Thermococcales*, consistent with the repeated presence of these organisms in enrichment culture libraries (see next section). Furthermore, four archaeal phylotypes recovered in samples from the 5 day deployment were found to group with *Thermoplasma acidophilum*, the closest cultivated relative of the euryarchaeal group II lineage (Reysenbach et al. 1997).

The occurrence of cultivable thermophiles and hyperthermophiles in low-temperature hydrothermal flu-

ids from the Juan de Fuca Ridge has been documented by Holden et al. (1998). These microorganisms were not detectable in ambient seawater, suggesting that they had grown in a warm, stable habitat below the seafloor where temperatures are permissive.

Positive enrichments of hyperthermophiles from cold plume waters shortly after eruption events (Huber et al. 1990; Delaney et al. 1998) also support the hypothesis of the existence of subcrustal habitats. The concept of a subsurface biosphere (Gold 1992; Deming & Baross 1993; Pedersen 1993) has been supported with recent demonstrations of several deep subsurface environments such as aquifers, groundwater from a borehole in granitic rock, marine sediments, and continental petroleum reservoirs (Ghiorse & Wilson 1988; Parkes et al. 1994; Szewzyk et al. 1994; L'Haridon et al. 1995) and now seems well-accepted in the scientific world.

Molecular ecology of high-temperature microbial communities

Cultivated thermophilic microorganisms

Enrichment culture isolation experiments performed with samples collected from deep-sea hydrothermal fluids and chimneys revealed the presence of a physiologically diverse microbial community and led to the characterization of numerous bacterial and archaeal thermophiles (and hyperthermophiles), including both chemolithoautotrophic and chemoorganoheterotrophic strains. Most of the microorganisms known to thrive in the hottest parts of the ecosystem are anaerobic hyperthermophiles (with optimal growth temperature above 80 °C), which fall into the domain *Archaea*. Representative species of both kingdoms of the *Archaea*, the *Crenarcheota* and the *Euryarchaeota*, have been isolated from deep-sea vents, but the predominant group is composed of heterotrophic sulfur metabolizers from the order *Thermococcales* that include the genera *Thermococcus* and *Pyrococcus* (for reviews, see Prieur et al. 1995, 1999).

The other *Archaea* isolated so far at great depths are the sulfate-reducer *Archaeoglobus profundus* and the sulfite-reducer *Archaeoglobus veneficus* (Burggraf et al. 1990; Huber et al. 1997), and chemolithotrophic methanogens of the genera *Methanococcus* (Jones et al. 1983, 1989; Zhao et al. 1988; Jeanthon et al. 1998, 1999a, b) and *Methanopyrus* (Kurr et al. 1991) within the *Euryarchaeota*. Most of these genera and species are not restricted to deep-sea vents. So far, *A. fulgidus*, *A. profundus*, *Thermococcus celer* and *T. litoralis* have been isolated from deep subterranean oil reservoirs and coastal hydrothermal systems (Stetter et al. 1993; L'Haridon et al. 1995; E. Bonch-Osmolovskaya, pers. comm.). Members of the kingdom *Crenarchaeota* isolated from deep-sea vents include a variety of sulfur-metabolizers consisting of strains of the genus *Desulfurococcus* (Jannasch et al. 1988), *Pyrodictium abyssi* (Pley et al. 1991) and *Staphylothermus marinus* (Fiala et al. 1986), and the facultatively aerobic obligate chemolithoautotroph *Pyrolobus fumarii* (Blöchl et al. 1997). This organism currently represents the life-form with the highest upper temperature limit for growth (113 °C).

Only a few thermophilic microorganisms belonging to the domain *Bacteria* have been identified from enrichment cultures. The occurrence in deep-sea hydrothermal fluids and chimneys of thermophilic aerobic bacteria from the genera *Bacillus* and *Thermus* has been reported (Marteinsson et al. 1995, 1996). Several fermentative strains assigned to the order *Thermotogales* (Marteinsson et al. 1997) and further identified as representatives of the species *Thermotoga maritima* and *Thermonanaerobacter ethanolicus* (Ollivier, pers. comm.), other strains described as a new species, *Thermosipho melanesiensis* (Antoine et al. 1997), and a chemolithoautotrophic sulfur reducing highly motile rod belonging to the new genus, *Desulfurobacterium* (L'Haridon et al. 1998a) complete the short list of bacterial representatives known to dwell in the deep vents.

Lipid analyses

Despite the effort involved in the isolation of new strains, description of new taxa, and physiological work, little is known about the distribution and relative abundance of individual vent inhabitants, and the dynamics and successional changes associated with the establishment of microbial communities. This is in part due to the difficulty in designing such experiments in deep-sea vents, and in part due to the limitations of enrichment culture technologies that have traditionally hampered microbial diversity studies.

The distribution of microorganisms in sediments, on and within hydrothermal vent chimneys from various hydrothermal vent sites has been assessed by using several techniques. Membrane lipids and their associated fatty acids have proved to be useful diagnostic biomarkers for determining bacterial diversity

and biomass levels in hydrothermal sediment samples collected from the Guaymas Basin (Holzer et al. 1988) and from vents at 13° N (Elsgaard et al. 1991). Polar lipid fatty acids (PLFA) have been validated as a measure of eubacterial and eukaryotic biomass whereas the presence of tetraether and the ratio diether to tetraether forms provide a definitive test for the occurrence of archaea in environmental samples (Hedrick et al. 1991).

Lipids were detected in all the sections of a flange structure from the Endeavor segment of the Juan de Fuca Ridge (Hedrick et al. 1992). Archaeal ether lipids were present in all but the external layer, with the highest concentrations in the middle layers. The ratio of diether to tetraether lipids increased markedly across the temperature gradient of the flange, that is, from the coolest to the hottest sections. The authors suggested that these lipid biomarker distribution patterns provide evidence for more than one strategy among the archaea in response to habitat variability. The concentration of total ether lipids in the flange samples ranged from 0.17 to 6.3 μg per g dry weight of flange solids. By using conversion factors, the population inhabiting the interior of the flange was estimated to vary from 10^6 to $>10^8$ cells per gram (Baross & Deming 1995).

The relative dominance of bacterial fatty acids in the external layers of an active chimney from the EPR (9°50′ N) and of archaeal ether lipids within the wall and in its internal parts has also been demonstrated by Chevaldonné (1996). On the basis of lipid concentrations, populations greater than 10^5 and ranging between 10^7–10^9 cells/g dry weight of chimney material were predicted for the bacterial and archaeal populations, respectively. Successful acridine orange direct counts on the two active chimneys from the MAR and from the EPR measured densities of microorganisms on the outer crust of about 10^9 to 10^{10} cells/g dry weight (Chevaldonné & Godfroy 1996). The densities dropped dramatically to about 10^5 cells/g within the chimney wall of the EPR chimney and did not reach the limit of detection of the method in the innermost part of the sulfide structure (about 10^3–10^4 cells/g).

Whole-cell hybridization studies

The microbial distribution in hydrothermal vent chimneys from the MAR was evaluated by the combined use of enrichment cultures and whole-cell hybridization with domain- and kingdom-specific probes (Harmsen et al. 1997a). By both methods, the largest numbers of microorganisms were found in the upper and outer parts of a chimney, although even its inner parts contained culturable and detectable numbers of cells. In most of the samples, different archaeal and bacterial morphotypes were observed (Figures 2a and 2b).

The highest numbers of culturable anaerobic thermophiles mainly represented by heterotrophic sulfur-reducing cocci (up to 3×10^6 cells per g dry weight of chimney) were measured in the upper part of the sulfide structure. These counts were consistent with the total numbers of cells enumerated by whole-cell hybridization in the same samples (3.9×10^7 cells per g dry weight). In the subsamples that contained high cell densities, the numbers of archaeal and bacterial cells were almost equivalent. When measurable, densities of crenarchaeal cells were generally very low. However, they were found to represented 19% of the archaeal community in the upper external part of the chimney sample. In the sampled chimneys, the fluid diffused through an axial zone of high porosity up to the surface and the sides of the structures. Therefore the growth of this type of structure occurs both horizontally and vertically. Surprisingly, bacterial cells were found to dominate in the inner parts of the structure that may be older and hotter than the outer walls.

Four newly designed oligonucleotide probes designed to detect most of the thermophilic members of the genus *Bacillus*, most species of the genus *Thermus*, the genus *Thermotoga* and the order *Aquificales* were used on cells extracted from the same chimneys (Harmsen et al. 1997b). Despite the reported presence of strains from the genera *Bacillus* and *Thermus* in deep-sea hydrothermal vents (Marteinsson et al. 1995, 1996), no positive signals could be obtained by using the probes specific to these genera. Only one sample contained cells that hybridized to the probe specific to the genera *Thermosipho* and *Thermotoga* thereby corroborating their occurrence in enrichment cultures (Harmsen et al. 1997a).

The combined use of the *Aquificales*-specific probe with a probe specific to the domain *Bacteria* (which did not hybridize with the known members of the order *Aquificales*) allowed Harmsen and his colleagues to quantify in MAR chimney samples morphologically diverse cells that hybridized with both probes (Figures 2c and 2d). These included small rods corresponding to the morphotype of a chemolithoautotrophic sulfur-reducer isolated from the same habitat that was

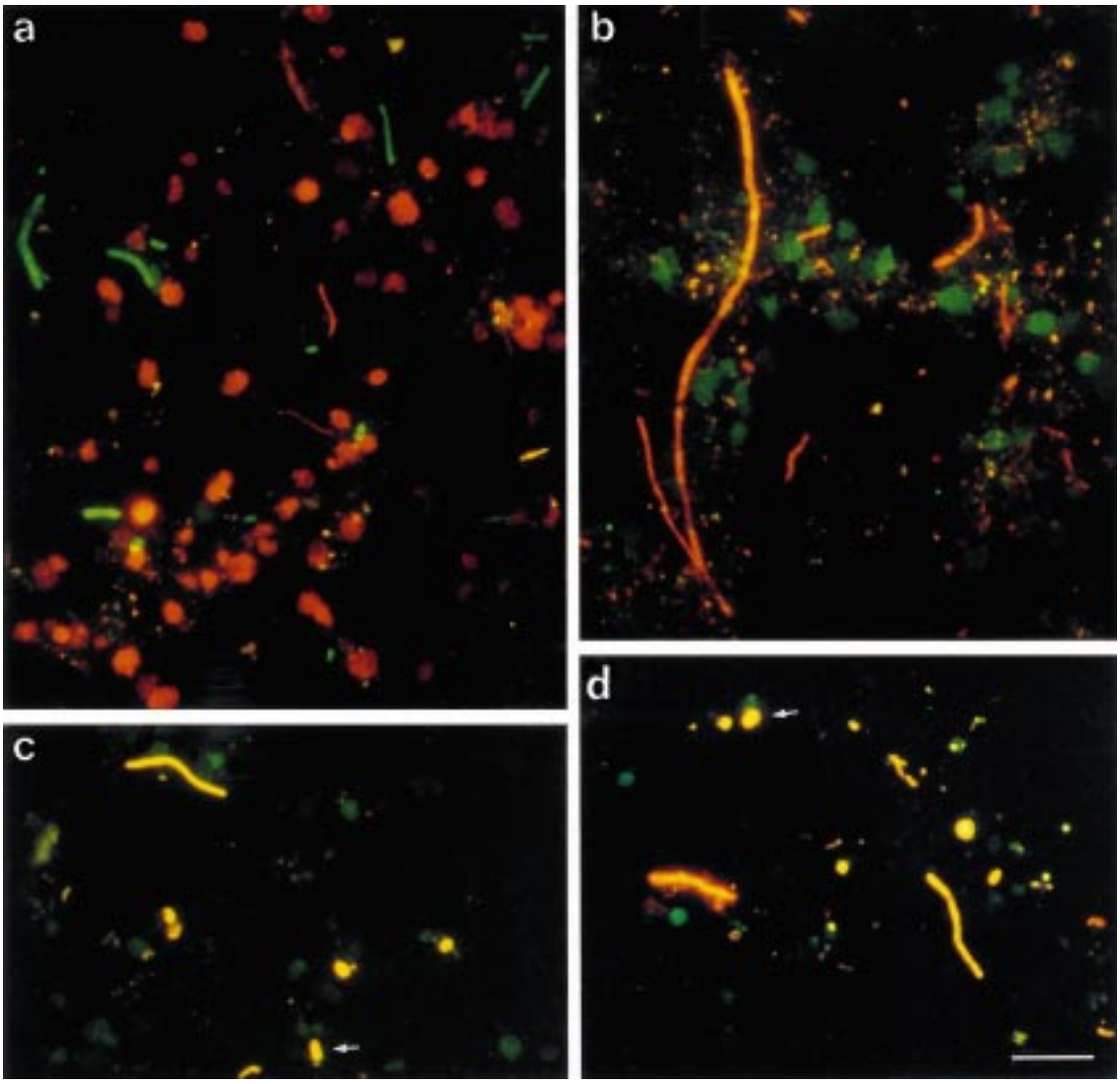

Figure 2. Whole-cell hybridizations of cells extracted from different chimney samples hybridized simultaneously with (A) fluorescein-labeled probe S-D-Bact-0338-a-A-18 (green fluorescence) and rhodamine-labeled probe S-D-Arch-0915-a-A-20 (red fluorescence), (B) rhodamine-labeled probe S-D-Bact-0338-a-A-18 and fluorescein-labeled probe S-D-Arch-0915-a-A-20, (C and D) fluorescein-labeled probes S-O-Hydr-0540-a-A-19 and S-D-Arch-0915-a-A-20 and rhodamine-labeled probe S-D-Bact-0338-a-A-18. Cells that hybridized with probes S-O-Hydr-0540-a-A-19 and probe S-D-Bact-0338-a-A-18 appear yellowish and are dominant. The cells with a morphotype similar to that of *Desulfurobacterium thermolithotrophum* are indicated by arrows. Specificities of the probes are reported in Table 1. Epifluorescence photomicrographs (double exposures) were taken. Magnification, $\times$1,125. Bar, 10 μm. With the permission of the publisher.

described as the new genus *Desulfurobacterium* within the order *Aquificales* (L'Haridon et al. 1998a).

Cells of *D. thermolithotrophum* were shown to contain sequences identical to the targets of the probe specific to the *Aquificales* and the probe specific to *Bacteria*. By using this diagnostic feature it was demonstrated that the cells sharing the same properties represented up to 40% of the bacterial population. To analyze the distribution of these autotrophic sulfur-reducers in geographically distant deep-sea vent sites, new isolates were obtained from several deep-sea hydrothermal sites (EPR, MAR, Guaymas Basin, North-Fiji Basin). Using amplified ribosomal DNA restriction analysis (ARDRA) (Vaneechoutte et al. 1992), it was found that most of them consituted new species (L'Haridon et al. 1998b). Analyses of their 16S rDNA sequences indicate that *D. thermolithotrophum* and its phylogenetically very closely related counterparts may

be members of a new family. Phylotypes that grouped within this putatively new family were also recovered in vent cap samples collected at MAR (Reysenbach et al. 1997). These findings suggest that these microorganisms are ubiquitous and abundant within deep-sea hydrothermal environments and that they may play a significant role in the sulfur cycle therein.

Several attempts have been made to extract DNA directly from chimneys or sulfide cores in order to provide new insights into the diversity of the microbial communities they harbour (Reysenbach et al. 1998; Corre et al., unpubl.). However, most extractions failed probably due to the high binding efficiency of the structure minerals and to the patchy distribution of communities in the sulfides (Hedrick et al. 1992; Harmsen et al. 1997a; Corre et al., unpubl.). Nethertheless these problems have been overcome in a few cases. Chevaldonné (1996) obtained measurable amounts of DNA from two chimneys sampled at the MAR (up to 80 μg of DNA per gram of wet chimney). Amplifiable DNA was also extracted successfully from freshly collected sulfide structures (Riley, pers. comm.) and is currently being used in the description of active thermophilic methanogenic communities and in the detection and identification of new strains within both of the known genera of thermophilic methanogens (*Methanococcus* and *Methanopyrus*) (Riley 1997).

Conclusions and future prospects

Since the initial discovery of deep-sea hydrothermal vents, our understanding of the various microbial processes occurring in this spatially and temporally variable environment is somewhat constrained by the relatively rare opportunities to obtain fresh and representative sample material (high cost and limited accessibility). Despite these substantial technical difficulties, progress on the isolation and physiological and taxonomic investigations of selected vent microbes (particularly thermophiles) has been remarkable. However, our perception of the existing microbial diversity of this ecosystem remains rudimentary. The challenge in attempting to approach as closely as possible the conditions of natural habitats in the laboratory is an important consideration to improve our accessibility to the as yet uncultivated microorganisms (Palleroni 1997). Improvements in culture methods would provide an exciting source of organisms for the microbiologist, but realizing an objective description of the community by only using culture methods is unlikely.

Despite their limitations, which have been recently extensively reviewed (Amann et al. 1995; von Wintzingerode et al. 1997; Head et al. 1998), the application of molecular techniques have generated advances that could not have been achieved using conventional techniques (Pace 1997; Hugenholtz et al. 1998). The rRNA-based methods are constantly being improved (Lebaron et al. 1997; Schönhuber et al. 1997; Marchesi et al. 1998) and the applicability of molecular biological approaches to other genes has been demonstrated (Osborn et al. 1993; Hodson et al. 1995; Ueda et al. 1995; Götz et al. 1996). However, the scope of such studies is often severely limited by the labour intensiveness of molecular methods which limit the number of samples that can be analyzed. Oligonucleotide microchips, originally developed for rapid sequence analysis of genomic DNA (Yershov et al. 1996), may allow large numbers of samples to be processed rapidly and in miniaturized format. Their application is being extended to the detection of microorganisms by hybridization of oligonucleotides with rRNA (Guschin et al. 1997). Since a wealth of rRNA and DNA sequences of functional genes, retrieved from many different species of microorganisms, are now available, the huge potential of the technique will have application not only for determinative microbiology but also to studies of global diversity.

The incongruity that remains between bacterial populations detected by cultivation and molecular retrieval approaches suggests that none of them can provide alone a complete and totally objective view of community composition and structure. The complementarity of their resolving power should prove useful to expand further our understanding of biological and functional diversity and the evolutionary processes that have led to it. Confirming this assumption, molecular techniques have been successfully used as guides to facilitate cultivation attempts (Huber et al. 1995) and to monitor and optimize enrichment cultures (Santegoeds et al. 1996; Burggraf et al. 1997; Ward et al. 1997, 1998).

Exploration and harvesting of hydrothermal microorganisms are necessary to expand our understanding of the geographic limits of microbial species (cosmopolitan or endemic) in these habitats. Too few analyses of microbial community composition have been attempted to reach conclusions about the distribution and the modes of dissemination of microbial species in

these environments. More generally, assessment of the microbial diversity in these biotopes will not only lead to the discovery of new and unusual microorganisms that would expand our knowledge in the evolution and functioning of the biosphere, but could also provide many economically valuable products and processes with wide ranging benefits.

Note added in proof

Recently, two key papers reported molecular phylogeneic analyses of archaeal communities in shallow marine vent waters (Takai & Sako 1999) and in deep-sea hydrothermal vent waters and chimneys (Takai & Horikoshi 1999).

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References

Amann RI, Krumholtz M & Stahl DA (1990) Fluorescent oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J. Bacteriol. 172: 762–770

Amann RI, Ludwig W & Schleifer KH (1995) Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol. Rev. 59: 143–169

Antoine E, Cilia V, Meunier JR, Guezennec J, Lesongeur F & Barbier G (1997) *Thermosipho melanesiensis* sp. nov., a new thermophilic anaerobic bacterium belonging to the order *Thermotogales*, isolated from deep-sea hydrothermal vents in the Southwestern Pacific Ocean. Int. J. Syst. Bacteriol. 47: 1118–1123

Baross JA & Deming JW (1995) Growth at high temperatures: isolation and taxonomy, physiology, ecology. In: Karl DM (Ed) The Microbiology of Deep-sea Hydrothermal Vents (pp 169–217). CRC Press Inc, Boca Raton

Bintrim SB, Donohue TJ, Handelsman J, Roberts GP & Goodman RM (1997) Molecular phylogeny of *Archaea* from soil. Proc. Natl. Acad. Sci. USA 94: 277–282

Blöchl E, Rachel R, Burggraf S, Hafenbradl D, Jannasch HW & Stetter KO (1997) *Pyrolobus fumarii*, gen. and sp. nov., represents a novel group of archaea, extending the upper temperature limit for life to 113 °C. Extremophiles 1: 14–21

Bowers TS, Campbell AC, Measures CI, Spivack AJ, Khadem M & Edmond JM (1988) Chemical controls on the composition of vent fluids at 13° N, 11° N and 21° N, East Pacific Rise. J. Geophys. Res. 93: 4522–4536

Burggraf S, Jannasch HW, Nicolaus B & Stetter KO (1990) *Archaeoglobus profundus*, sp. nov., represents a new species within the sulfate-reducing archaebacteria. System. Appl. Microbiol. 13: 24–28

Burggraf S, Mayer T, Amann R, Schadhauser S, Woese CR & Stetter KO (1994) Identifying members of the domain *Archaea* with rRNA-targeted oligonucleotide probes. Appl. Environ. Microbiol. 60: 3112–3119

Burggraf S, Heyder P & Eis N (1997) A pivotal Archaea group. Nature 385: 780

Byers HK, Patel B & Stackebrandt E (1997) Design and application of four oligonucleotide probes specific for *Thermus* species. System. Appl. Microbiol. 20: 248–254

Cary SC & Giovannoni SJ (1993) Transovarial inheritance of endosymbiotic bacteria in clams inhabiting deep-sea hydrothermal vents and cold seeps. Proc. Natl. Acad. Sci. USA 90: 5695–5699

Cary SC, Warren W, Anderson E & Giovannoni SJ (1993) Identification and localization of bacterial endosymbionts in hydrothermal vent taxa with symbiont-specific polymerase chain reaction amplification and in situ hybridization techniques. Mar. Mol. Biol. Biotechnol. 2: 51–62

Cary SC, Cottrell MT, Stein JL, Camacho F & Desbruyères D (1997) Molecular identification and localization of filamentous symbiotic bacteria associated with the hydrothermal vent annelid *Alvinella pompejana*. Appl. Environ. Microbiol. 63: 1124–1130

Cary SC, Shank T & Stein J (1998) Worms bask in extreme temperatures. Nature 391: 545–546

Cavanaugh CM, Wirsen CO & Jannasch HW (1992) Evidence for methylotrophic symbionts in a hydrothermal vent mussel (Bivalvia: Mytilidae) from the Mid-Atlantic Ridge. Appl. Environ. Microbiol. 58: 3799–3803

Chandler DP, Fredrickson JK & Brockman FJ (1997) Effect of PCR template concentration on the composition and distribution of total community 16S rDNA clone libraries. Mol. Ecol. 6: 475–482

Chevaldonné P, Desbruyères D & Childress JJ (1992) Some like it hot ... and some even hotter. Nature 359: 593–594

Chevaldonné P (1996) Ecologie des cheminées hydrothermales actives. Doctoral Thesis, Université de la Méditerrannée, Marseille, 257 pp

Chevaldonné P & Godfroy A (1996) Enumeration of microorganisms from deep-sea hydrothermal vent chimneys. FEMS Microbiol. Lett. 146: 211–216

Childress JJ & Fisher CR (1992) The biology of hydrothermal vent animals: Physiology, biochemistry, and autotrophic symbioses. Oceanogr. Mar. Biol. Annu. Rev. 30: 337–441

Corliss JB, Dymond J, Gordon LI, Edmond JM, Von Herzen RP, Ballard RD, Green K, Williams D, Bainbridge A, Crane K & van Andel TH (1979) Submarine thermal springs on the Galapagos Rift. Science 203: 1073–1083

Corre E, Reysenbach AL & Prieur D (1997) Molecular phylogenetic analysis of the microbial diversity of a vent cap sample. First International Symposium on Deep-sea Hydrothermal Vent Biology. p 81

Delaney JR, Kelley DS, Milley MD, Butterfield DA, Baross JA, Wilcock WSD, Embley RW & Summit M (1998) The quantum event of oceanic crustal accretion: impacts of diking at Mid-Ocean Ridges. Science 281 222–230

DeLong EF (1992) Archaea in coastal marine environments. Proc. Natl. Acad. Sci. USA 89: 5685–5689

DeLong EF, Wu KY, Prezelin BB & Jovine RVM (1994) High abundance of archaea in Antarctic marine picoplankton. Nature 371: 695–697

Desbruyères D, Chevaldonné P, Alayse-Danet AM, Jollivet D, Lallier FH, Jouin-Toulmond C, Zal F, Sarradin PM, Cosson R, Caprais JC, Arndt C, O'Brien J, Guezennec J, Hourdez S, Riso R, Gaill F, Laubier L & Toulmond A (1998) Biology and ecology of the 'Pompei worm' (*Alvinella pompejana* Desbruyères et Laubier), a normal dweller of an extreme deep-sea environment: A synthesis of current knowledge and recent developments. Deep-Sea Res. II 45: 383–422

Deming JW & Baross JA (1993) Deep-sea smokers: Windows to a subsurface biosphere. Geochim. Cosmochim. Acta 57: 3219–3230

Distel DL, Lane DJ, Olsen GJ, Giovannoni SJ, Pace B, Pace NR, Stahl DA & Felbeck H (1988) Sulfur-oxidizing bacterial endosymbionts: analysis of phylogeny and specificity by 16S rRNA sequences. J. Bacteriol. 170: 2506–2510

Distel DL, Felbeck H & Cavanaugh CM (1994) Evidence for phylogenetic congruence among sulfur-oxidizing chemoautotrophic bacterial endosymbionts and their bivalve hosts. J. Mol. Evol. 38: 533–542

Distel DL, Lee HKW & Cavanaugh CM (1995) Intracellular coexistence of methano- and thioautotrophic bacteria in a hydrothermal vent mussel. Proc. Natl. Acad. Sci. USA 92: 9598–9602

Durand P, Reysenbach AL, Prieur D & Pace N (1993) Isolation and characterization of *Thiobacillus hydrothermalis* sp. nov., a mesophilic obligately chemolithotrophic bacterium isolated from a deep-sea hydrothermal vent in Fiji Basin. Arch. Microbiol. 159: 39–44

Durand P, Benyagoub A & Prieur D (1994) Numerical taxonomy of heterotrophic sulfur-oxidizing bacteria isolated from southwestern Pacific hydrothermal vents. Can. J. Microbiol. 40: 690–697

Edwards DB & Nelson DC (1984) DNA-DNA hybridization studies of the bacterial symbionts of hydrothermal vent tube worms (*Riftia pachyptila* and *Tevnia jerichonana*). Appl. Environ. Microbiol. 57: 1082–1088

Elsgaard L, Guezennec J, Benbouzid-Rollet N & Prieur D (1991) Fatty acid composition of sulfate-reducing bacteria isolated from deep-sea hydrothermal vents. Kiel Meeresforsch. Sonderh. 8: 182–187

Elsgaard L, Isaksen MF, Jorgensen BB, Alayse AM & Jannasch HW (1994) Microbial sulfate reduction in deep-sea sediments at Guaymas Basin hydrothermal vent area: influence of temperature and substrates. Geochim. Cosmochim. Acta 58: 3335–3343

Elsgaard L, Guezennec J, Benbouzid-Rollet N & Prieur D (1995) Mesophilic sulfate-reducing bacteria from three deep-sea hydrothermal vent sites. Oceanologica Acta 8: 95–104

Farrelly V, Rainey FA & Stackebrandt E (1995) Effect of genome size and rrn gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. Appl. Environ. Microbiol. 61: 2798–2801

Fiala G, Stetter KO, Jannasch HW, Langworthy TA & Madon J (1986) *Staphylothermus marinus* sp. nov., represents a novel genus of extremely thermophilic submarine heterotrophic archaebacteria growing up to 98 °C. Syst. Appl. Microbiol. 8: 106–113

Fuhrman JA, McCallum K & Davis AA (1992) Novel major archaebacterial group from marine plankton. Nature 356: 148–149

Gaill F, Desbruyères D & Prieur D (1987) Bacterial communities associated with 'Pompei worms' from the East Pacific Rise hydrothermal vents: SEM, TEM observations. Microbial Ecol. 13: 129–139

Gebruk AV, Pimenov NV & Savvichev AS (1993) Feeding specialization of bresiliid shrimps in the TAG site hydrothermal community. Mar. Ecol. Prog. Ser. 98: 247–253

Ghiorse WC & Wilson JT (1988) Microbial ecology of the terrestrial biosphere. Adv. Appl. Microbiol. 33: 107–172

Giovannoni SJ, Turner S, Olsen GJ, Barns S, Lane DJ & Pace NR (1988) Evolutionary relationships among cyanobacteria and green chloroplasts. J. Bacteriol. 170: 3584–3592

Godon JL, Zumstein E, Dabert P, Habouzit F & Moletta R (1997) Molecular microbial diversity of an anaerobic digestor as determined by small-subunit rDNA sequence analysis. Appl. Environ. Microbiol. 63: 2802–2813

Gold T (1992) The deep, hot biosphere. Proc. Natl. Acad. Sci. USA 89: 6045–6049

Götz A, Pukall R, Smit E, Tietze E, Prager R, Tschäpe H, Van Elsas JD & Smalla K (1996) Detection and characterization of broad-host range plasmids in environmental bacteria by PCR. Appl. Environ. Microbiol. 62: 2621–2628

Guschin DY, Mobarry BK, Proudnikov D, Stahl DA, Rittmann BE & Mirzabekov AD (1997) Oligonucleotide microchips as genosensors for determinative and environmental studies in microbiology. Appl. Environ. Microbiol. 63: 2397–2404

Haddad A, Camacho F, Durand P & Cary SC (1995) Phylogenetic charaterization of the epibiotic bacteria associated with the hydrothermal vent polychaete *Alvinella pompejana*. Appl. Environ. Microbiol. 61: 1679–1687

Harmsen HJM, Prieur D & Jeanthon C (1997a) Distribution of microorganisms in deep-sea hydrothermal vent chimneys investigated by whole-cell hybridization and enrichments of thermophilic subpopulations. Appl. Environ. Microbiol. 63: 2876–2883

Harmsen HJM, Prieur D & Jeanthon C (1997b) Group-specific 16S rRNA-targeted oligonucleotide probes to identify thermophilic bacteria in marine hydrothermal vents. Appl. Environ. Microbiol. 63: 4061–4068

Head IM, Saunders JR & Pickup RW (1998) Microbial evolution, diversity and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms. Microbial Ecol. 35: 1–21

Hedrick DB, Guckert JB & White DC (1991) Archaebacterial ether lipid diversity analyzed by supercritical fluid chromatography: integration with a bacterial lipid protocol. J. Lipid Res. 32: 659–666

Hedrick DB, Pledger RD, White DC & Baross JA (1992) *In situ* microbial ecology of hydrothermal vent sediments. FEMS Microbiol. Rev. 101: 1–10

Hershberger KL, Barns SM, Reysenbach AL, Dawson SC & Pace NR (1996) Wide diversity of Crenarchaeota. Nature 384: 420

Hodson RE, Dustman WA, Garg RP & Moran MA (1995) *In situ* PCR for vizualization of microscale distribution of specific genes and gene products in prokaryotic communities. Appl. Environ. Microbiol. 61: 4074–4082

Holden JF, Summit M & Baross JA (1998) Thermophilic and hyperthermophilic microorganisms in 3–30 °C hydrothermal fluids following a deep-sea volcanic eruption. FEMS Microbiol. Ecol. 25: 33–41

Holzer GU, Kelly PJ & Jones WJ (1988) Analysis of lipids from a hydrothermal vent methanogen and associated vent sediment by supercritical fluid chromatography. J. Microbiol. Methods 8: 161–173

Huber H, Jannasch H, Rachel R, Fuchs T & Stetter KO (1997) *Archaeoglobus veneficus* sp. nov., a novel facultative chemolithoautotrophic hyperthermophilic sulfite reducer, isolated from abyssal black smokers. Syst. Appl. Microbiol. 20: 374–380

Huber R, Stoffers P, Cheminee JL, Richnow HH & Stetter KO (1990) Hyperthermophilic archaebacteria within the crater and

open-sea plume of erupting Macdonald Seamount. Nature 345: 179–182

Huber R, Burggraf S, Mayer T, Barns SM, Rossnagel P & Stetter KO (1995) Isolation of a hyperthermophilic archaeum predicted by in situ RNA analysis. Nature 376: 57–58

Hugenholtz P, Goebel BM & Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J. Bacteriol. 180: 4765–4774

Jannasch HW, Wirsen CO, Nelson DC & Robertson LA (1985) *Thiomicrospira crunogena* sp. nov., a colorless, sulfur-oxidizing bacterium from a deep-sea hydrothermal vent. Int. J. Syst. Bacteriol. 35: 422–424

Jannasch HW, Wirsen CO, Molyneaux SJ & Langworthy TA (1988) Extremely thermophilic fermentative archaebacteria of the genus *Desulfurococcus* from deep-sea hydrothermal vents. Appl. Environ. Microbiol. 54: 1203–1209

Jannasch HW, Nelson DC & Wirsen CO (1989) Massive natural occurrence of unusually large bacteria (*Beggiatoa* sp.) at a hydrothermal vent site. Nature 342: 834–836

Jannasch HW (1995) Microbial interactions with hydrothermal fluids. In: Humphris SE, Zierenberg RA, Mullineaux LS & Thomson RE (Eds) Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions (pp 273–296). Geophysical monograph 91, American Geophysical Union, Washington DC

Jeanthon C & Prieur D (1990) Susceptibility to heavy metals and characterization of heterotrophic bacteria isolated from two hydrothermal vent polychaetes, *Alvinella pompejana* and *Alvinella caudata*. Appl. Environ. Microbiol. 56: 3308–3314

Jeanthon C (1991) Bactéries hétérotrophes associées aux Alvinellidae inféodés aux sources hydrothermales profondes (13° N): comportement vis-à-vis des métaux lourds. Doctoral Thesis, Université de Bretagne Occidentale, Brest, 126 pp

Jeanthon C, Mergeay M, Diels L & Prieur D (1991) Susceptibility to heavy metals and antibiotics of eight deep-sea hydrothermal vent bacteria carrying a 51.7 kb plasmid. Kiel Meeresforsch. Sonderh. 8: 193–197

Jeanthon C, L'Haridon S, Reysenbach AL, Vernet M, Messner P, Sleytr UW & Prieur D (1998) *Methanococcus infernus* sp. nov., a novel hyperthermophilic lithotrophic methanogen isolated from a deep-sea hydrothermal vent. Int. J. Syst. Bacteriol. 48: 913–919

Jeanthon C, L'Haridon S, Reysenbach AL, Corre E, Vernet M, Messner P, Sleytr UW & Prieur D (1999a) *Methanococcus vulcanius* sp. nov., a novel hyperthermophilic methanogen isolated from East Pacific Rise and identification of *Methanococcus* sp. DSM 4213 as *Methanococcus fervens* sp. nov. Int. J. Syst. Bacteriol. 49: 583–589

Jeanthon C, L'Haridon S, Pradel N & Prieur D (1999b) Rapid identification of hyperthermophilic methanococci isolated from deep-sea hydrothermal vents. Int. J. Syst. Bacteriol. 49: 591–594

Jones WJ, Leigh JA, Mayer F, Woese CR & Wolfe RS (1983) *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. Arch. Microbiol. 136: 254–261

Jones WJ, Stugard CE & Jannasch HW (1989) Comparison of thermophilic methanogens from submarine hydrothermal vents. Arch. Microbiol. 151: 214–318

Jorgensen BB, Isaksen MF & Jannasch HW (1992) Bacterial sulfate reduction above 100 °C in deep-sea hydrothermal vent sediments. Science 258: 1756–1757

Jurgens G, Lindström K & Saano A (1997) Novel group within the kingdom Crenarchaeota from boreal forest soil. Appl. Environ. Microbiol. 63: 803–805

Karl DM (1995) Ecology of free-hydrothermal vent microbial communities. In: Karl DM (Ed) The Microbiology of Deep-sea Hydrothermal Vents (pp 35–124). CRC Press Inc, Boca Raton

Kurr M, Huber R, König H, Jannasch HW, Fricke H, Trincone A, Kristjansson JK & Stetter KO (1991) *Methanopyrus kandleri* gen. and sp. nov. represents a novel group of hyperthermophilic methanogens, growing at 110 °C. Arch. Microbiol. 156: 239–247

Lane DJ, Pace B, Olsen GJ, Stahl D, Sogim ML & Pace NT (1985) Rapid determination of 16S ribosomal RNA sequences for phylogenetic analysis. Proc. Natl. Acad. Sci. USA 82: 6955–6969

Lebaron P, Catala P, Fajon C, Joux F, Baudart J & Bernard L (1997) A new, sensitive, whole-cell hybridization technique for detection of bacteria involving a biotinylated oligonucleotide probe targeting rRNA and tyramide signal amplification. Appl. Environ. Microbiol. 63: 3274–3278

L'Haridon S, Reysenbach AL, Glénat P, Prieur D & Jeanthon C (1995) Hot subterranean biosphere in a continental oil reservoir. Nature 377: 223–224

L'Haridon S, Cilia V, Messner P, Raguénès G, Gambacorta A, Sleytr UW, Prieur D & Jeanthon C (1998a) *Desulfurobacterium thermolithotrophum* gen. nov., sp. nov., a novel autotrophic, sulfur-reducing bacterium isolated from a deep-sea hydrothermal vent. Int. J. Syst. Bacteriol. 48: 701–711

L'Haridon S, Speck M, Vetriani C, Reysenbach AL, Prieur D & Jeanthon C (1998b) The *Desulfurobacteriaceae*, a new family of thermophilic bacteria from deep-sea hydrothermal vents. Thermophiles 98' International Conference BP 28

MacGregor BJ, Moser DP, Alm EW, Nealson KH & Stahl DA (1997) Crenarchaeota in Lake Michigan sediment. Appl. Environ. Microbiol. 63: 1178–1181

Maidak BL, Olsen GJ, Larsen N, Overbeek R, McCaughey MJ & Woese CR (1997) The RDP (Ribosomal Database Project). Nucleic Acids Res. 25: 109–110

Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ & Wade WG (1998) Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl. Environ. Microbiol. 64: 795–799

Marteinsson VT, Birrien JL, Kristjansson JK & Prieur D (1995) First isolation of thermophilic aerobic non-sporulating heterotrophic bacteria from deep-sea hydrothermal vents. FEMS Microbiol. Ecol. 18: 163–174

Marteinsson VT, Birrien JL, Jeanthon C & Prieur D (1996) Numerical taxonomic study of thermophilic *Bacillus* isolated from three geographically separated deep-sea hydrothermal vents. FEMS Microbiol. Ecol. 21: 255–266

Marteinsson VT, Birrien JL & Prieur D (1997) *In situ* enrichment and isolation of thermophilic microorganisms from deep-sea hydrothermal environments. Can. J. Microbiol. 43: 694–697

McInerney JO, Wilkinson M, Patching JW, Embley TM & Powell R (1995) Recovery and phylogenetic analysis of novel archaeal rRNA sequences from a deep-sea deposit feeder. Appl. Environ. Microbiol. 61: 1646–1648

Michard GF, Albarède A, Michard JF, Minster J, Charlou L & Tan N (1984) Chemistry of solutions from the 13° N East Pacific Rise hydrothermal site. Earth Planet Sci. Lett. 67: 297–307

Moyer CL, Dobbs FC & Karl DM (1994) Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. Appl. Environ. Microbiol. 60: 871–879

Moyer CL, Dobbs FC & Karl DM (1995) Phylogenetic diversity of the bacterial community from a microbial mat at an active, hy-

drothermal vent system, Loihi Seamount, Hawaii. Appl. Environ. Microbiol. 61: 1555–1562

Moyer CL, Tiedje JM, Dobbs FC & Karl DM (1998) Diversity of deep-sea hydrothermal vent *Archaea* from Loihi Seamount, Hawaii. Deep-Sea Res. II 45: 303–317

Munson MA, Nedwell DB & Embley TM (1997) Phylogenetic diversity of Archaea in sediment samples from a coastal salt marsh. Appl. Environ. Microbiol 63: 4729–4733

Muyzer G, Teske A, Wirsen CO & Jannasch HW (1995) Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Arch. Microbiol. 164: 165–172

Nelson DC (1989) Characterization of large, autotrophic *Beggiatoa* spp. abundant at hydrothermal vents of the Guaymas Basin. Appl. Environ. Microbiol. 55: 2909–2917

Nelson DC & Fisher CR (1995) Chemoautotrophic and methanotrophic endosymbiotic bacteria at deep-sea vents and seeps. In: Karl DM (Ed) The Microbiology of Deep-sea Hydrothermal Vents (pp 125–167). CRC Press Inc, Boca Raton

On SLW, Vandamme PAR & Ursing JB (1998) International Committee on Systematic Bacteriology. Subcommittee on the taxonomy of *Campylobacter* and related bacteria. Int. J. Syst. Bacteriol. 48: 619–620

Olsen GJ, Lane DJ, Giovannoni SG, Pace NR & Stahl DA (1986) Microbial ecology and evolution: a ribosomal RNA approach. Annu. Rev. Microbiol. 40: 337–365

Osborn AM, Bruce KD, Strike P & Ritchie DA (1993) Polymerase chain reaction-restriction length polymorphism analysis shows divergence among *mer* determinants from Gram-negative soil bacteria indistinguishable by DNA-DNA hybridizations. Appl. Environ. Microbiol. 59: 4024–4030

Pace NR (1997) A molecular view of microbial diversity and the biosphere. Science 276: 734–740

Pace NR, Stahl DA, Lane DJ & Olsen GJ (1986) The analysis of natural microbial populations by ribosomal RNA sequences. Adv. Microbiol. Ecol. 9: 1–55

Palleroni NJ (1997) Prokaryotic diversity and the importance of culturing. Antonie van Leeuwenhoek 72: 3–19

Parkes RJ, Cragg BA, Bale SJ, Getliff JM, Goodman K, Rochelle PA, Fry JC, Weightmann AJ & Harvey SM (1994) Deep bacterial biosphere in Pacific Ocean sediments. Nature 371: 410–413

Pedersen K (1993) The deep subterranean biosphere. Earth Sci. Rev. 34: 243–260

Pley U, Schipka J, Gambacorta A, Jannasch HW, Fricke H, Rachel R & Stetter KO (1991) *Pyrodictium abyssi*, sp. nov. represents a novel heterotrophic marine archaeal hyperthermophile archaeon growing at 110 °C. System Appl. Microbiol. 14: 245–253

Polz MF & Cavanaugh CM (1995) Dominance of one bacterial phylotype at a Mid-Atlantic Ridge hydrothermal vent site. Proc. Natl. Acad. Sci. USA 92: 7232–7236

Polz MF & Cavanaugh CM (1998) Bias in template-to-products ratios in multitemplate PCR. Appl. Environ. Microbiol. 64: 3724–3730

Polz MF, Robinson JJ, Cavanaugh CM & Van Dover CL (1998) Trophic ecology of massive shrimp aggregations at a Mid-Atlantic Ridge hydrothermal vent site. Limnol. Oceanogr. 43: 1631–1638

Pond DW, Bell MV, Dixon DR, Fallick AE, Segonzac M & Sargent JR (1998) Stable-carbon-isotope composition of fatty acids in hydrothermal vent mussels containing methanotrophic and thiotrophic bacterial endosymbionts. Appl. Environ. Microbiol. 64: 370–375

Preston CM, Wu KY, Molinski TF & DeLong EF (1996) A psychrophilic crenarchaeon inhabits a marine sponge: *Crenarchaeum symbiosum* gen. nov., sp. nov. Proc. Natl. Acad. Sci. USA 93: 6241–6246

Prieur D & Jeanthon C (1987) Preliminary study of heterotrophic bacteria isolated from two deep-sea hydrothermal vent invertebrates: *Alvinella pompejana* (Polychaete) and *Bathymodiolus thermophilus* (Bivalve). Symbiosis 4: 97–98

Prieur D, Chamroux S, Durand P, Erauso G, Fera P, Jeanthon C, Le Borgne L, Mével G & Vincent P (1990) Metabolic diversity in epibiotic microflora associated with the Pompeii worms *Alvinella pompejana* and *A. caudata* (Polychaetae: Annelida) from deep-sea hydrothermal vents. Mar. Biol. 106: 361–367

Prieur D, Jeanthon C & Erauso G (1995) Hyperthermophilic life at deep-sea hydrothermal vents. Planet Space Science 43: 115–122

Prieur D, Voytek M, Reysenbach AL & Jeanthon C (1999) Thermophilic microorganisms in the deep-sea. In: Reysenbach AL, Voytek M & Mancinelli R (Eds) Biodiversity, Ecology and Evolution of Thermophiles in Yellowstone National Park. Plenum Publishing, NY (in press)

Raguénès G, Pignet P, Gauthier G, Peres A, Christen R, Rougeaux H, Barbier G & Guezennec J (1996) Description of a new polymer-secreting bacterium from a deep-sea hydrothermal vent, *Alteromonas macleodii* subsp. *fijiensis*, and preliminary characterization of the polymer. Appl. Environ. Microbiol. 62: 67–73

Raguénès GHC, Peres A, Ruimy R, Pignet P, Christen R, Moaec M, Rougeaux H, Barbier G & Guezennec J (1997a) *Alteromonas infernus* sp. nov., a new polysaccharide-producing bacterium isolated from a deep-sea hydrothermal vent. J. Appl. Microbiol. 82: 422–430

Raguénès G, Christen R, Guezennec J, Pignet P & Barbier G (1997b) *Vibrio diabolicus* sp. nov., a new polysaccharide-secreting organism isolated from a deep-sea hydrothermal vent polychaete annelid, *Alvinella pompejana*. Int. J. Syst. Bacteriol. 47: 989–995

Raskin L, Stromley JM, Rittman BE & Stahl DA (1994) Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. Appl. Environ. Microbiol. 60: 1232–1240

Rau GH (1981) Hydrothermal vent clam and tube worm $^{13}C/^{12}C$: further evidence of nonphotosynthetic food sources. Science 213: 338–340

Rau GH & Hedges JI (1979) Carbon-13 depletion in a hydrothermal vent mussel: suggestion of a chemosynthetic food source. Science 203: 648–649

Reysenbach AL, Wickham GS & Pace NR (1994) Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park. Appl. Environ. Microbiol. 60: 2113–2119

Reysenbach AL, Kirshtein J, Corre E. & Prieur D (1997) *In situ* growth chambers deployed at deep-sea vents: providing clues to novel archaeal and bacterial diversity. First International Symposium on Deep-sea Hydrothermal Vent Biology. p 76

Reysenbach AL, Holm NG, Hershberger K, Prieur D & Jeanthon C (1998) In search of a subsurface biosphere at a slow-spreading ridge. In: Herzig PM, Humphris SE, Miller DJ & Zierenberg RA (Eds) Proc. ODP Sci. Results, Vol 158 (pp 355–360), College Station, TX

Riley PW (1997) The development of an alternative to 16S rDNA, the *mcrA* gene, as a phylogenetic tool in the description of methanogen communities in the marine environment. First International Symposium on Deep-sea Hydrothermal Vent Biology. p 109

Ruby EG & Jannasch HW (1982) Physiological characteristics of *Thiomicrospira* sp. strain L-12 isolated from deep-sea hydrothermal vents. J. Bacteriol. 149: 161–165

Ruby EG, Wirsen CO & Jannasch HW (1981) Chemolithotrophic sulfur-oxidizing bacteria from the Galapagos Rift hydrothermal vents. Appl. Environ. Microbiol. 42: 317–324

Santegoeds CM, Nold SC & Ward DM (1996) Denaturing gradient gel electrophoresis used to monitor the enrichment culture of aerobic chemoorganotrophic bacteria from a hot spring cyanobacterial mat. Appl. Environ. Microbiol. 62: 3922–3928

Sayler GS & Layton AC (1990) Environmental application of nucleic acid hybridization. Annu. Rev. Microbiol. 44: 625–648

Schleper C, Holben W & Klenk HS (1997) Recovery of crenarchaeotal ribosomal DNA sequences from freshwater-lake sediments. Appl. Environ. Microbiol. 63: 321–323

Schönhuber W, Fuchs B, Juretschko S & Amann R (1997) Improved sensitivity of whole-cell hybridization by the combination of horseradish peroxidase-labeled oligonucleotides and tyramide signal amplification. Appl. Environ. Microbiol. 63: 3268–3273

Stahl DA, Lane DJ, Olsen GJ & Pace NR (1984) Analysis of hydrothermal vent-associated symbionts by ribosomal RNA sequences. Science 224: 409–411

Stahl DA & Amann R (1991) Development and application of nucleic acid probes. In: Stackebrandt E & Goodfellow M (Eds) Nucleic Acid Techniques in Bacterial Systematics (pp 205–248). John Wiley & Sons, Chichester

Stetter KO, Huber R, Blöchl E, Kurr M, Eden RD, Fielder M, Cash H & Vance I (1993) Hyperthermophilic archaea are thriving in deep North Sea and Alaskan oil reservoirs. Nature 356: 743–745

Stetter KO (1996a) Hyperthermophilic prokaryotes. FEMS Microbiol. Rev. 18: 149–158

Stetter KO (1996b) Hyperthermophiles in the history of life. In: Bock GR & Goode JA (Eds) Evolution of Hydrothermal Ecosystems on Earth (and Mars?) (pp 1–18). John Wiley & Sons, Chichester

Suzuki M & Giovannoni SJ (1996) Bias caused by template annealing in the amplification mixtures of 16S rRNA genes by PCR. Appl. Environ. Microbiol. 62: 625–630

Szewzyk U, Szewzyk R & Stenström TA (1994) Thermophilic, anaerobic bacteria isolated from a deep borehole in granite in Sweden. Proc. Natl. Acad. Sci. USA 91: 1810–1813

Takai K & Horikoshi K (1999) Genetic diversity of archaea in deep-sea hydrothermal vents environments. Genetics 152: 1285–1297

Takai K & Sako Y (1999) A molecular view of archaeal diversity in marine and terrestrial hot water environments. FEMS Microbiol. Ecol. 28: 177–188

Tornabene TG (1985) Lipid analysis and the relationship to chemotaxonomy. Methods Microbiol. 18: 209–234

Torsvik T, Torsvik V, Keswani J & Whitman WB (1993) Oligonucleotide probes to 16S-rRNA of methanogenic bacteria. International Workshop on Molecular Biology and Biotechnology of Extremophiles and Archaebacteria. Wako, Japan. pp 131

Ueda T, Suga Y & Matsuguchi T (1995) Molecular phylogenetic analysis of a soil mircrobial community in a soybean field. Eur. J. Soil Sci. 46: 415–421

Ueda T, Suga Y, Yahiro N & Matsuguchi T (1995) Remarkable N_2-fixing bacterial diversity detected in rice roots by molecular evolutionary analysis of *nif*H gene sequences. J. Bacteriol. 177: 1414–1417

Van Dover CL, Fry B, Grassle JF, Humphris S & Rona PA (1988) Feeding biology of the shrimp *Rimicaris exoculata* at hydrothermal vents on the Mid-Atlantic Ridge. Mar. Biol. 98: 209–216

Vandamme P, Falsen E, Rossau R, Hoste B, Segers P, Tytgat R & De Ley (1991) Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy: emendation of descriptions and proposal of *Arcobacter* gen. nov. Int J. Syst. Bacteriol. 41: 88–103

Vaneechoutte M, Rossau R, De Vos P, Gillis M, Janssens D, Paepe, N, De Rouck A, Fiers T, Claeys G & Kersters K (1992) Rapid identification of bacteria of the *Comanonadaceae* with amplified ribosomal DNA-restriction analysis (ARDRA). FEMS Microbiol. Lett. 93: 227–234

Vetriani C, Reysenbach AL & Doré J (1998) Recovery and phylogenetic analysis of archaeal rRNA sequences from continental shelf sediments. FEMS Microbiol. Lett. 161: 83–88

Vincent P, Pignet P, Talmont F, Bozzi L, Fournet B, Guezennec J, Jeanthon C & Prieur D (1994) Production and characterization of an exopolysaccharide excreted by a deep-sea hydrothermal vent bacterium isolated from the polychaete annelid *Alvinella pompejana*. Appl. Environ. Microbiol. 60: 4134–4141

Von Damm KL, Edmond JM, Grant B, Measures CI, Walden B & Weiss RF (1985a) Chemistry of submarine hydrothermal solutions at 21° N, East Pacific Rise. Geochim. Cosmochim. Acta. 49: 2197–2220

Von Damm KL, Edmond JM, Measures CI & Grant B (1985b) Chemistry of submarine hydrothermal solutions at Guaymas Basin, Gulf of California. Geochim. Cosmochim. Acta. 49: 2221–2237

von Wintzingerode F, Göbel UB & Stackebrandt E (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol. Rev. 21: 213–229

Ward DM, Bateson MM, Weller R & Ruff-Roberts AL (1992) Ribosomal RNA analysis of microorganisms as they occur in nature. Adv. Microbiol. Ecol. 12: 219–286

Ward DM, Santegoeds CM, Nold SC, Ramsing NB, Ferris MJ & Bateson MM (1997) Biodiversity within hot spring microbial mat communities: molecular monitoring of enrichment cultures. Antonie van Leeuwenhoek 71: 143–150

Ward DM, Ferris MJ, Nold SC & Bateson MM (1998) A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. Microbiol. Mol. Biol. Rev. 62: 1353–1370

Wirsen CO & Jannasch HW (1978) Physiological and morphological observations on *Thiovulum* sp. J Bacteriol 136: 765–774

Wirsen CO, Brinkhoff T, Kuever J, Muyzer G, Molyneaux S & Jannasch HW (1998) Comparison of a new *Thiomicrospira* strain from the Mid-Atlantic Ridge with known hydrothermal vent isolates. Appl. Environ. Microbiol. 64: 4057–4059

Woese CR (1987) Bacterial evolution. Microbiol. Rev. 51: 221–271

Yamamoto H, Hiraisi A, Kato K, Chiura HX, Maki Y & Shimizu A (1998) Phylogenetic evidence for the existence of novel thermophilic bacteria in hot spring sulfur-turf microbial mats in Japan. Appl. Environ. Microbiol. 64: 1680–1687

Yershov G, Barsky V, Belgovskiiy A, Kirillov E, Kreindlin E, Ivanov I, Parinov S, Guschin D, Drobishev A, Dubiley S & Mirzabekov A (1996) DNA analysis and diagnostics on oligonucleotide microchips. Proc. Natl. Acad. Sci. USA 93: 4913–4918

Zhao H, Wood AG, Widdel F & Bryant MP (1988) An extremely thermophilic *Methanococcus* from a deep-sea hydrothermal vent and its plasmid. Arch. Microbiol. 150: 178–183