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## THERMOPHILES FROM DEEP-SEA HYDROTHERMAL VENTS

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THERMOPHILES  
HYDROTHERMAL VENT  
ARCHAEA  
THERMOSTABILITY

**ABSTRACT.** – Deep-sea hydrothermal vents represent extreme environments where original microbial communities have evolved during geological times. Of particular interest are the thermophilic and hyperthermophilic microbes thriving in the various microhabitats determined by the geochemical processes at work in the hydrothermal vent fields. In the present review, the general characteristics of the hydrothermal vents as habitats for thermophiles and hyperthermophiles are described. The microbial diversity is discussed on the basis of molecular and cultivation approaches. The most original features of thermophily at molecular level are briefly reviewed. Data gathered during the last two decades have substantially modified our understanding of diversity and evolution of deep-sea hydrothermal vent communities and ecology.

THERMOPHILES  
SOURCES HYDROTHERMALES  
ARCHAEA  
THERMOSTABILITÉ

**RÉSUMÉ.** – Les sources hydrothermales profondes constituent des environnements extrêmes où des communautés bactériennes originales se sont développées au cours des temps géologiques. Les thermophiles et hyperthermophiles colonisant les divers microhabitats déterminés par les processus géochimiques prévalant dans ces milieux présentent un intérêt particulier. Les caractéristiques principales de ces habitats sont passées en revue. Les connaissances sur la diversité microbienne, estimées à la fois par les approches moléculaires et de culture, sont discutées. Les aspects les plus originaux de l'adaptation à la thermophilie sont brièvement décrits. Les données accumulées au cours des deux dernières décades ont considérablement modifié notre compréhension de la diversité et de l'évolution des communautés microbiennes des sources hydrothermales profondes et de leur écologie.

### INTRODUCTION

Since the discovery of microbial life in Yellowstone hot springs by Thomas Brock more than 30 years ago, it has become more and more evident that life is present in a great variety of extreme environments. In an anthropocentric view, most of these extreme environments were unsuitable for microbial life and this discovery opened the way to a new era for microbiologists. Inventorying *a priori* hostile environments demonstrated that most of them were not only able to tolerate some sporadic microbial forms of life, but constitute the selective habitats of a great variety of microorganisms belonging to the 2 domains defined by Woese (1977): Archaea and Bacteria. These microorganisms have been called 'extremophiles'. Considering the parameters controlling their optimal growth, they are named thermophiles (>60°C), hyperthermophiles

(>80°C), psychrophiles, acidophiles, alcalophiles, barophiles or halophiles. Not only do they tolerate extreme environments but these extreme conditions are required for proper growth of microorganisms that have been selected over geologic time scale. Generally, they cannot grow in conditions suitable for eukaryotic forms of life. Following the work of Brock on terrestrial hot springs and thermophiles (Brock 1969), the discovery of deep-sea vents at the end of the 1970s boosted the research of the most extreme microorganisms. Among the different teams contributing to the research on extremophiles biodiversity, a remarkable contribution to the description of new genera and new species of hyperthermophiles is due to Stetter and his colleagues at University of Regensburg, Germany (Stetter 1998, Stetter 1999a,b). Besides these recent reviews, the present one focuses more specifically on hyperthermophiles from deep-sea hydrothermal vents.



### ***Hydrothermal vents as habitats for thermophiles and hyperthermophiles***

Thermophiles and hyperthermophiles have been isolated from various habitats: terrestrial hot springs, solfataras and volcanic acidic hot springs, shallow marine hot springs, submarine vents, deep-sea sediments and deep-sea hydrothermal vents. Moreover, evidence of the existence of microorganisms in the deep terrestrial and oceanic subsurface has been increasing during the last decade (Delaney *et al.* 1998, Summit & Baross 2001). Since the temperature and pressure of subsurface environments increase with depth, they are potentially among the most appropriate habitats for hyperthermophiles. An exhaustive list of thermophiles and hyperthermophiles isolated from these various environments as well as related references can be found in a recent review (Amend & Shock 2001) and on a dedicated web site at <http://levee.wustl.edu/~chan/Research/research.html>.

Geochemical mechanisms at work on mid-oceanic ridges result from the interaction between seawater and volcanic rocks and from seawater and hydrothermal fluxes of heat and dissolved matter. Since their discovery in 1977 at the Galapagos Spreading Center (GSC) at 2500 m water depth (Hessler & Smithey 1983), it has been shown that seafloor hydrothermal vents constitute, in almost every mid-oceanic ridges investigated, ecosystems supporting important biomass and high productivity in contrast with other deep sea environments. Faunal assemblages, similar to those discovered at GSC, have been sampled at various sites on the East Pacific Rise (EPR) (Lutz & Kennish 1993), Juan de Fuca Ridge (Tunnicliffe 1988), Mid-Atlantic Ridge (MAR) (Van Dover 1995), and the spreading centres of Western and South-Western Pacific Back Arc Basin (Desbruyères *et al.* 1994, Hessler *et al.* 1987). Numerous active sites are still under investigation along the mid-oceanic ridges in the Pacific Ocean, the Atlantic Ocean and more recently in the Indian Ocean (Fig. 1). Intensive research has been conducted on different vent fields at East Pacific Rise from 26°S to 21°N and at Mid-Atlantic Ridge between 15 and 40°N. Currently eight vent fields have been described from MAR, including Mount Saldanha (36°N) discovered in 1998 during the Portuguese-French SALDANHA cruise (Desbruyères *et al.* 2000). Most known hydrothermal vents along the mid-oceanic ridges are located on young crust where the cooling of hot basaltic material drives hydrothermal flow (Fornari & Embley 1995). Recently an off-axis hydrothermal vent field named "Lost City" has been discovered near the Mid-Atlantic Ridge at 30°N at nearly 15 km from the spreading axis on a 1.5 Myr old crust, demonstrating that hydrothermal venting occurs not only along mid-oceanic ridges, but also on old regions of the oceanic crust away from spread-

ing centres (Kelley *et al.* 2001). These last findings indicate that a much larger portion of the oceanic crust may support hydrothermal activity and microbial life than previously thought. On mid-oceanic ridges, at depth ranging from 1 500 to 4 000 m, hydrothermal fluids can reach temperatures up to 400°C whereas ambient sea water is around 2°C, producing steep temperature gradients within diffusion structures. Hydrothermal solutions reaching the seafloor contain various products resulting from hot acidic seawater and volcanic rocks: metals (Fe, Mn, Zn, Cu, etc.) and reduced sulphur (Zierenberg *et al.* 2000). Most of the hydrothermal fluids display physico-chemical compositions that are unsuitable for eukaryotic organisms due to their high toxicity. However, active chimneys (white and black smokers) are densely populated, hosting not only microorganisms belonging to Bacteria and Archaea, but also a very rich macrofauna typical of these environments (Desbruyères & Segonzac 1997). Strikingly, fluids of "Lost City" off-axis vent field were found to be relatively cool (40–75°C) and alkaline (pH 9.0–9.8), supporting dense microbial communities including anaerobic thermophiles, but rare macrofaunal assemblages that typify most vent environments (Kelley *et al.* 2001).

Hydrothermal vent ecosystems stability is directly dependent upon the variations in fluid fluxes and chemical composition of the fluids. Microbial blooms are observed at the initiation of magmatic cycle, suggesting that the upper oceanic crust is inoculated with microbial communities (Zierenberg *et al.* 2000). Volcanic events initially are followed by relative increase in fluid temperature and magmatic gases, often accompanied by decrease salinity in hydrothermal fluids as a result of supercritical phase separation in seawater in the subsurface. The high-salinity components of the phase separated fluids reaches the seafloor a few years later (Von Damm 1995). Direct observation of discontinuity in vent emission has been done in different ridge dynamic contexts (Desbruyères *et al.* 2000). High variability in fluid composition at a same vent according to volcanic activity has been reported. In some cases, like at Lucky Strike (MAR 37°N), variability in chemical composition suggests the existence of two different sources (Von Damm *et al.* 1998). As an example, the concentration of most of the major chemical components in MAR vent fluid end-members is given in table I (Desbruyères *et al.* 2000). Despite the important differences between sites, it can be noticed that H<sub>2</sub>S, CO<sub>2</sub>, NH<sub>4</sub> and CH<sub>4</sub> are present in vent fluids and are available as primary compounds for chemolithoautotrophic microbes essential to the good running of the ecosystems. Another source of variability between different vent fields, acting as a major ecological factor, is the particulate (mineral and organic) content of the fluids. Finally, these



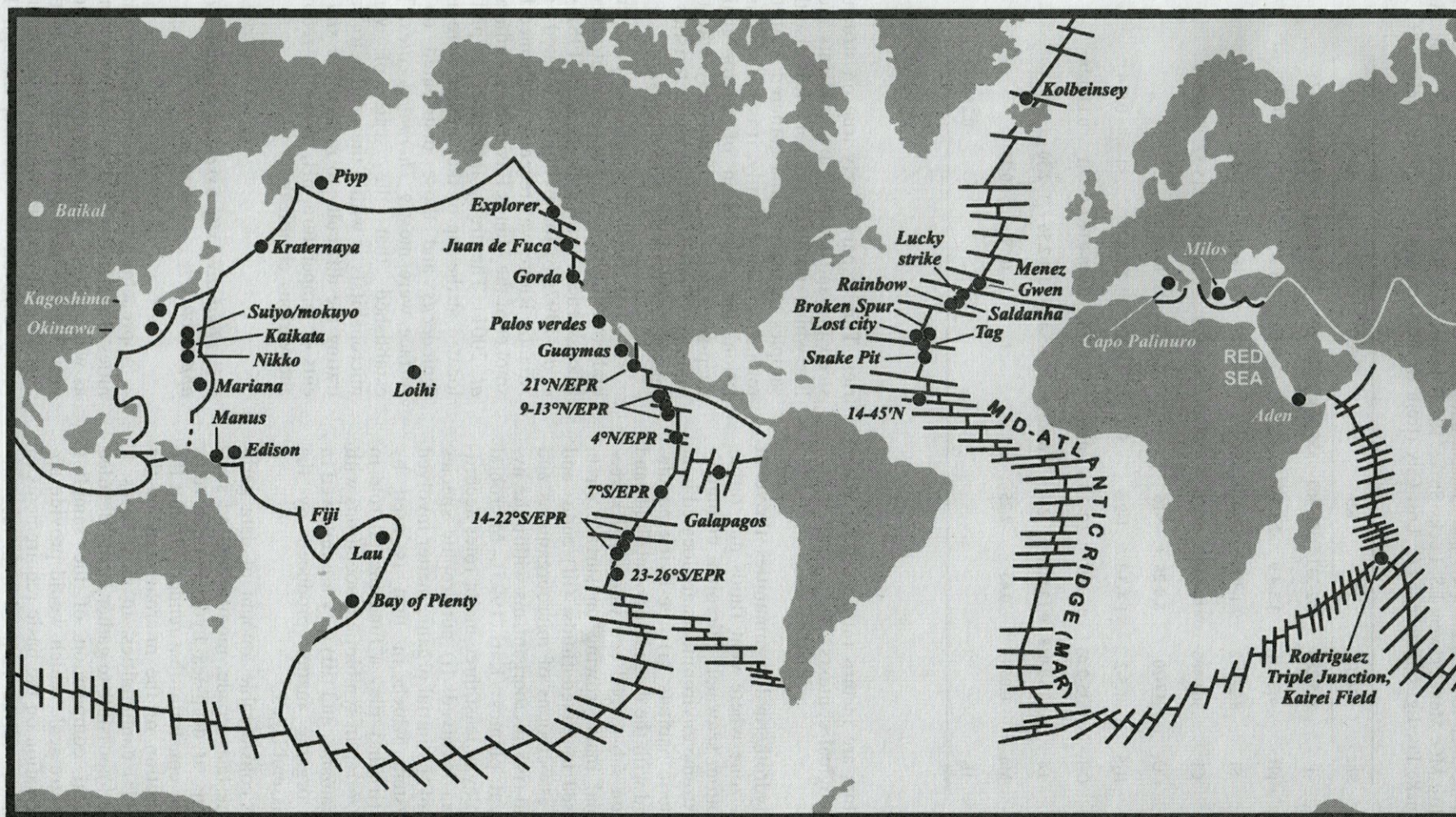


Fig. 1. – Distribution of deep-sea hydrothermal vents along oceanic ridges (from Desbruyères & Ségonzac 1997 modified and completed).



Table I. – End-member temperature (°C) and concentrations of chemical species (mmol kg<sup>-1</sup>) for the different M.A.R. vent fields: MG: Menez Gwen; LS: Lucky Strike; Rb: Rainbow; BS: Broken Spur; T.A.G.: Trans Atlantic Geotraverse; SP: Snake Pit; Lg: Logatchev; LC: Lost City (from Desbruyères 2000 completed Kelley 2001).

Site	MG	LS	Rb	BS	T.A.G.	SP	Lg	LC
T	265-284	152-333	360-365	356-364	270-363	335-356	>353	40-75
pH	4.2-4.8	3.5-4.9	2.8-3.1		2.5-3.4	3.7-3.9	<3.3	9-9.8
Si	8.2-11.2	9.1-17.5	6.9-8.0		18-22	18-20	7-8.2	9-19
Cl <sup>-</sup>	360-400	410-540	>750	469	633-675	550-563	515-522	546-549
CO <sub>2</sub>	17-20	8.9-28	<16		2.9-4.1	10.63		
H <sub>2</sub> S	1.5-2	1.4-3.3	1-2.5	9.3	2.5-6.7	2.7-6.1	<1	0.064
CH <sub>4</sub>	1.35-2.63	0.5-0.97	2.2-2.5	0.065	0.14-0.62	0.046-0.062	2.1	0.13-0.28
Fe	0.002-0.018	0.13-0.86	24	1.68-2.16	1.64-5.45	1.8-2.56	2.50	
Mn	0.068	0.45	2.25	0.26	1	0.49	0.33	
H <sub>2</sub>								0.25-0.43

groups of factors are in turn influenced by water depth and geological contexts.

Deep-sea hydrothermal communities thrive in the interfacial zone where vent fluids mix turbulently with bottom seawater. Microbial communities in these extreme environments are located in at least four generic habitats: (i) free-living populations associated with the discharged vent fluids and depending upon sub-seabed ecosystems, (ii) free-living microbial mats growing on surface strata that are exposed to mixed fluids, (iii) endo- and exosymbiotic associations of microorganisms and vent fauna, and (iv) microorganisms within the hydrothermal vent plumes (Karl 1995). Microbial communities play at least three major roles according to the taxa involved: (i) autotrophic species produce most of the initial organic matter involved in the ecosystem which in turn is used by heterotrophs, (ii) they play a fundamental role in detoxification, often in symbiotic associations with eukaryotic organisms, and (iii) they are involved in recycling of organic matter produced by the macrofaunal assemblages.

Despite the considerable amount of data gathered during the last decade, monitoring and sampling conditions at deep-sea hydrothermal vents are still a major constraint that render difficult the accurate description of the microhabitats of the various microbial communities. In most cases, it remains almost impossible to gather *in situ* detailed physico-chemical composition of the sampling sites at centimetre scale which would provide appropriate information on the basic requirements of the microorganisms further isolated in the laborato-

ries. This is particularly true on structures where steep pH and temperature gradients are observed like white and black smokers. It is also the case for samples of macrofauna gathered from these environments. Consequently, it is not surprising if isolation and cultivation of microorganisms from deep-sea vents are still the only way to describe their basic physiological characteristics and to infer their role in the ecosystems.

The first report of occurrence of discrete microbial communities using enrichment procedures and fluorescence *in situ* hybridisation (FISH) (Harmsen *et al.* 1997a,b) for a chimney located on the Mid-Atlantic Ridge was recently confirmed for a black smoker of the Manus Basin (New Guinea) (Takai *et al.* 2001). The archaeal phylotypes predominantly located at the top of the smoker were affiliated to *Ignicoccus* and those distributed on the vent surface were mostly *Thermococcales*. These data established that the scale of variation of microhabitats is within the centimetre range, illustrating the difficulty to measure *in situ* the gradients of temperature, pH, oxidation-redox potential and various chemicals.

#### **Microbial diversity assessed by molecular approach**

Approaching the diversity of microbial communities associated with deep-sea hydrothermal vents, as well as from other ecosystems, has long been a very tedious task since only two methods were available: direct microscopic observation and laboratory cultivation. Evidence, through 16S rDNA



sequences, of numerous uncultured microorganisms in natural communities has gained strength during the last decade since pioneering work in that field (Ward *et al.* 1990, Ward *et al.* 1992). Despite specific bias introduced by molecular methods, demonstrated by the possible lack within the pool of amplified 16S rDNA of sequences corresponding to already isolated microorganisms, it is generally admitted that no more than 1 to 5% of the total microorganisms of a natural community are available through isolation procedures. Consequently, the molecular approach is complementary to the standard enrichment and cultivation procedures in assessing the diversity of microbial communities in extreme environment where our knowledge of appropriate isolation and culture techniques is still limited. This approach, based on extraction of total DNA from crude samples, amplification by PCR of 16S rDNA genes by use of universal primers for Bacteria and Archaea, cloning of these genes for isolation prior sequencing, has allowed, besides determination of microbial communities *in situ*, the discovery of entirely new phylogenetic lineages of prokaryotes (Moyer *et al.* 1995, Moyer *et al.* 1998, Takai & Horikoshii 1999). These novel taxa, based on FISH experiments, appear to be major components of the deep-sea vent ecosystems. Microbial mats are widely distributed at most deep-sea hydrothermal vent sites from Guaymas Basin to East Pacific Rise and Mid-Atlantic Ridge. Recently new phylotypes belonging to a group of uncultured *ε-Proteobacteria* have been identified using an *in situ* growth chamber at a MAR hydrothermal vent (Reysenbach *et al.* 2000). The same research group established on a different hydrothermal vent site located on the Southern East Pacific Rise that more than 98% of the sequences of 16S rDNA obtained from a sample of microbial mat were closely related to the previously identified *ε-Proteobacteria*. They concluded from these data and from previous indications that this new clade of *ε-Proteobacteria* may be endemic and widely distributed among deep-sea hydrothermal vents (Longnecker & Reysenbach 2001). Two recent results reinforce this hypothesis. Firstly, DGGE profiles and phylogenetic analysis, conducted on enrichment cultures of samples of hydrothermal fluids, chimney-like structures and tubes and specimens of *Alvinella* spp. collected on a deep-sea vent at 13°N EPR, demonstrated that archaeal sequences were related to the genus *Thermococcus* and the bacterial sequences to uncultured *ε-Proteobacteria*, and *Deferribacter thermophilus*, *Bacillus halodurans*, *Ralstonia pickettii* and *Marinitoga camini* (Slobodkin *et al.* 2001). Secondly, we demonstrated (unpublished results) by ARDRA profiles and phylogenetic analysis, conducted on enrichment cultures, using complex proteinaceous substrates, from white tubes of *Alvinella* spp. collected on 13°N EPR, that most archaeal sequences were related to the *Thermococcus* genus as previously

mentioned and that the bacterial sequences belonged to *Clostridiales* (20%) and *ε-Proteobacteria* (30%).

The diversity of metabolic types within the *ε-Proteobacteria* already isolated and cultivated from other environments makes it difficult to hypothesise about the metabolic capabilities of the various strains inhabiting deep-sea vents. Considering the high prevalence of the uncultured *ε-Proteobacteria* in both 16S rDNA gene populations of crude samples and of enrichment cultures and their putative role in sulphur cycling in deep-sea vents environments hypothesised by Longnecker *et al.*, inferred from the white sulphur-like coloration of the mat filaments, it is of considerable interest to succeed in culturing them in order to assess their basic metabolic properties.

#### *Cultivated thermophilic microorganisms from deep-sea hydrothermal vents*

Research in this field had two main incentives. Firstly, thermophilic and more generally extremophilic microorganisms represent an unprecedented source of enzymes displaying original properties interesting many industrial activities, and known as extremozymes. Hyperthermostable DNA polymerases from *Thermococcus* and *Pyrococcus* genera such as Deep-Vent from *Pyrococcus* sp. GB-D (New England Biolabs, Boston, USA) and Tfu Pol and Pab Pol respectively from *Thermococcus fumicolans* (Cambon Bonavita *et al.* 2000) and *Pyrococcus abyssi* (QbioGene, Illkirch, F) are examples of extremozymes. Despite the fact that extremozymes properties do not generally fit exactly the industry requirements, extremozymes are a matchless material for directed evolution and protein engineering programs. The second major incentive is related to molecular evolution and the origin of life question. Phylogenetic analyses, based on comparisons of 16S rDNA sequences, place the hyperthermophiles as the most slowly evolving of all forms of life, the first to have diverged from the last common universal ancestor. This suggests that life first emerged on this planet in hyperthermophilic conditions. This statement represents a controversial dogma (Wiegel & Adams 1998) that strongly fuels research on hyperthermophiles and deep-sea hydrothermal vents.

Within the Archaea inhabiting deep-sea hydrothermal vents are found the most extreme of known hyperthermophiles with growth temperature optima above 100°C. *Pyrolobus fumarii*, a facultatively aerobic chemolithoautotroph isolated from chimney walls MAR (Blöchl *et al.* 1997) with an optimum growth temperature of 106°C, measurable growth up to 113°C and ability to remain viable after one hour treatment in the autoclave at 121°C,



has the highest cardinal temperatures for any known microorganism. Next to *P. fumarii* are found the methanogen *Methanopyrus kandleri*, isolated from chimney walls of a black smoker where it reaches numbers of  $10^8$  per gram of chimney rock (Kurr *et al.* 1991), the obligate anaerobic heterotroph isolated from the Guaymas Basin *Pyrodictium abyssi* (Pley *et al.* 1991) and the barophilic chemoorganotrophic *Pyrococcus* sp. ES4 isolated from Juan de Fuca Ridge (Pledger & Baross 1991) which are able to grow up to 110 °C.

As free living microorganisms, the *Thermococcaceae* appear largely distributed on the various hydrothermal fields whether in shallow water or in the deep-sea. Strains belonging to this family are strictly anaerobic hyperthermophilic fermentative sulphur reducing heterotrophs. They use a variety of carbon substrates such as peptides and/or carbohydrates and are probably involved in recycling organic matter produced by autotrophs and higher organisms belonging to deep-sea vent communities. They are the predominant hyperthermophilic group of microorganisms isolated from deep-sea vents. Three genera are represented: *Paleococcus* with only one species, *Pyrococcus* (3 from the deep-sea among 5 valid species) and *Thermococcus* (10 species from the deep-sea among 21). Optimal temperatures for growth are at between 80–88 °C for *Paleococcus* and *Thermococcus* and between 90–95 °C for *Pyrococcus*. Differentiation of the species within the *Thermococcus* genus requires DNA-DNA hybridisation since they are morphologically and physiologically rather similar to each other. Some of them are barophilic with a direct effect of pressure on growth, like for *Paleococcus ferrophilus* (1–600 bar range, optimum at 300 bar) (Takai *et al.* 2000), *Pyrococcus abyssi* (optimum growth temperature 96 °C at 1 bar, 100 °C at 200 bars) (Erauso *et al.* 1993). *Thermococcus barophilus*, isolated from MAR at a depth of 3550 m on the external layer of a chimney wall and from enrichment cultures conducted at 400 bar, requires a minimum of 150–170 bar for growth in the range of 95–100 °C (Marteinsson *et al.* 1999b).

Hyperthermophilic methanogens are also well distributed in deep-sea hydrothermal vent environments. Besides *Methanopyrus kandleri* already mentioned, the genus *Methanococcus* has been documented from MAR (*M. infernus* (Jeanthon *et al.* 1998)), from EPR (*M. vulcanius* (Jeanthon *et al.* 1999)) and from Guaymas Basin, Gulf of California (*M. jannaschii* (Jones *et al.* 1983), *M. fervens* (Jeanthon *et al.* 1999)). Obligate anaerobic, they are chemolithotrophs. They use  $H_2$  and  $CO_2$  as the only substrate for growth and methane production. In the presence of  $CO_2$  and  $H_2$ , they reduce elemental sulphur to hydrogen sulphide.

The other Archaea isolated from deep-sea hydrothermal vents are the sulphate reducer

*Archaeoglobus profundus* (Burggraf *et al.* 1990), the sulphite reducer *Archaeoglobus veneficus* (Huber *et al.* 1997) and members of the kingdom *Crenarchaeota* including sulphur metabolisers from the genus *Desulfurococcus*, *Staphylothermus* (Fiala *et al.* 1986) and *Ignicoccus* (Huber *et al.* 2000).

Many of these genera and species are also found in other environments characterised by their extreme temperatures. *Thermococcus litoralis* (Neuner *et al.* 1990) initially isolated from a shallow marine hot spring was also isolated from solfatara and subterranean oil reservoirs. The genus *Archaeoglobus* is also found in Alaskan and North Sea oil reservoirs (Stetter *et al.* 1993) as well as from coastal submarine hot springs in Vulcano Island (Stetter 1988). Genera *Thermococcus*, *Archaeoglobus* and to a less extent *Methanococcus* appear to be ubiquitous in marine thermal habitats (Holden *et al.* 2001).

Comparatively, only a limited number of species belonging to the Bacteria domain have been isolated from deep-sea hydrothermal vents. Among described species of the Thermotogales *Thermosipho melanesiensis* (Antoine *et al.* 1997) was isolated from gills of a deep-sea hydrothermal mussel from the Lau Basin in South Western Pacific Ocean, *Thermosipho japonicus* isolated from the Iheya Basin of Okinawa (Takai & Horikoshi 2000). *Marinitoga camini* (optimum growth temperature of 55 °C) a chemoorganotrophic sulphur reducing strain (Wery *et al.* 2001a) and *Caloranaerobacter azorensis*, an anaerobic thermophilic bacterium belonging to the cluster XII of the Clostridiales (Wery *et al.* 2001b) were isolated from a chimney sample collected at MAR. A chemolithoautotrophic sulphur reducing Bacteria, *Desulfurobacterium thermolithotrophum*, with an optimum growth temperature of 70 °C, was isolated from a deep-sea hydrothermal chimney sample collected at the MAR (L'Haridon *et al.* 1998). More recently our laboratory has succeeded in culturing an  $\epsilon$ -Proteobacteria, *Caminibacter hydrogenophilus* (Alain *et al.* in press), proposed as the representative strain of a new family of  $\epsilon$ -Proteobacteria well distributed in deep-sea hydrothermal vent environments as reported by several authors (Longnecker & Reysenbach 2001, Reysenbach *et al.* 2000), on the basis of molecular approaches of microorganisms diversity. This strain was isolated from fragments of chimney rocks and emptied white tubes of *Alvinella pompejana* samples, collected at EPR 13°N. It represents a novel thermophilic, anaerobic, obligate hydrogen-oxidising bacterium and is able to grow chemolithoautotrophically under an atmosphere of  $H_2/CO_2$  with  $S^0$  or  $NO_3^-$  as electron acceptor and is probably involved in sulphur cycling in deep-sea hydrothermal vent ecosystems.

Aerobic thermophilic strains are less frequently isolated from deep-sea vent environments, proba-



bly due to anaerobic prevalent conditions above 35°C and to unbalanced efforts to uncover new species. However, aerobic Bacteria isolated from vents at 2000 m depth from the Guaymas Basin and MAR at 3500 m and belonging to the genera *Bacillus* and *Thermus* have been reported (Marteinsson *et al.* 1995, Marteinson *et al.* 1999a).

### *Molecular adaptations to thermophily*

Studies on life at high temperatures are comparatively recent and, despite the results gathered during the last two decades, remain a promising field of interest for topics as diverse as 'origin of life', biomolecules (in)stability, biotechnology and extremozymes, strategies for detection of novel hyperthermophilic organisms in extreme environments on Earth and preparing sampling for life discovery on other planets. All cell components of extremophiles microorganisms have to be heat adapted or at least heat resistant for periods of time compatible with corresponding metabolic constraints. This implies that not only proteins are stable at temperatures above 80, 100 or even 110°C according to the species considered, but that many low molecular weight metabolites and coenzymes, nucleic acids and lipids are either heat resistant or protected against denaturation and/or degradation by specific mechanisms. The upper limit of life at high temperatures evidenced from isolated Archaea might be slightly revised in the future. However, it depends on the stability of biomolecules, availability of energy and thermodynamics, and not the least on the ability of cells to maintain an appropriate proton permeability range of membranes in order to keep electrochemical proton gradients for energy gain (Albers *et al.* 2000).

Different recent reviews deal with the stability of proteins at high temperatures (Daniel & Cowan 2000, Jaenicke 2000, Ladenstein & Antranikian 1998, Scandurra *et al.* 2000). A surprising conclusion emerges from these studies: there are no general rules to achieve protein stabilisation. Each extremophilic protein adopts various strategies and the outstanding adaptation to extreme temperature and solvent conditions is realised through the same weak electrostatic and hydrophobic interactions among the ordinary amino acid residues which are also responsible for the proper balance between protein stability and flexibility in mesophilic proteins. Comparative studies between mesophilic enzymes and their hyperthermophilic counterparts indicate that hyperthermostability is gained through a variable combination of a small number of noncovalent features: minimisation of the surface energy and the hydration of apolar surface groups, increased compactness with a decrease of internal cavities and a high number of ionic interactions. The adaptation of proteins to extreme temperatures

appears then to be the result of a compromise between the increased rigidity responsible for thermal stability and the flexibility required for playing their physiological roles. Besides these intrinsic stability factors, protein stability can be enhanced by molecular chaperonins as demonstrated with the thermosome characterised from *Methanopyrus kandleri* (Andra *et al.* 1998). This property is probably critical at the upper temperature border of life where heat-shock proteins could play a key role: cultures of *P. fumarii* with the thermosome fully induced were able to survive 1 h autoclaving at 2 bar and 121°C (Blöchl *et al.* 1997). Recently, it has been shown that when overexpressed in *E. coli* the recombinant small heat shock protein (sHSP) from the hyperthermophile *Pyrococcus furiosus* (involved in adaptation to exposure to temperatures over growth temperature optimum), prevented the majority of *E. coli* proteins from aggregating *in vitro* for up to 40 min at 105 °C. Apparently, the sHSP confers a survival advantage on mesophilic Bacteria by preventing protein aggregation at supraoptimal temperatures (Laksanalamai *et al.* 2001). For more detail, the reader is directed to a recent review on the mechanisms for thermostability of hyperthermophilic enzymes (Vieille & Zeikus 2001).

High temperatures require also a tight control of exchanges at cytoplasmic membrane level. The membrane lipids, as basic components of the thermophilic microbial cell membrane, play a key role in thermophily. The ion permeability of the membrane increases with temperature and is a major factor determining the maximum growth temperature. As demonstrated by Könings, the most important finding is that the proton permeability of most bacterial and all archaeal membranes at their temperature of growth is maintained within a narrow window ( $H^+$  permeability coefficient near  $10^{-9} \text{ cm.s}^{-1}$ ). In contrast, the permeability of the membranes for sodium ions at different growth temperatures was not constant, but was found to increase exponentially with temperature in a similar way for all organisms studied (Albers *et al.* 2000). Changes in lipid composition of membrane is a common strategy of Bacteria and Archaea in response to changes of ambient temperatures. The 'core' lipids of the Archaea are mainly based on saturated isoprenoid chains linked to a glycerol backbone by ether bonds. The increase in cyclization of transmembrane lipids contributes to a better adaptation to high temperatures by reducing membrane fluidity (Gliozzi *et al.* 1983). Increase in growth temperatures of *M. jannaschii* induces an increase in the ratio tetraether/diether lipids. The resulting cyclization of the chains tends to decrease the motion of the lipids and therefore contributes to an acceptable membrane fluidity at elevated growth temperature (Sprott *et al.* 1991).



The maintenance of the genomes and more generally of nucleic acids of hyperthermophiles inhabiting deep-sea hydrothermal vents is also a challenging question since to high temperatures is added a variety of chemical compounds with possible genotoxic effects. Considerable research has been done during the last two decades to analyse the strategies of hyperthermophilic Bacteria and Archaea in DNA and RNA maintenance and summarised in a recent review (Daniel & Cowan 2000). The main problem of DNA stability at high temperatures is thermal degradation due to depurination and subsequent breakage of the phosphodiester bonds (Marguet & Forterre 1994). The DNA of *P. furiosus* was found to be 20 times more resistant to thermal degradation than DNA of *E. coli* on the basis of the number of DNA backbone breaks after incubation of the cells at 105 °C (Peak *et al.* 1995). Less is known about strategies developed to cope with the other harsh conditions found in these environments where the relative instability of fluids composition and of the vents themselves with time imply appropriate molecular adaptations. The case of an other extremophile, *Deinococcus radiodurans*, isolated from canned meat exposed to X rays is of great interest. The complete sequencing of its genome (White *et al.* 1999) demonstrated that all systems for DNA repair, DNA damage export, desiccation and starvation recovery, and genetic redundancy are present in one cell. However, a subsequent analysis of this genome reached the conclusion that the fundamental questions underlying the extreme resistance phenotype of *D. radiodurans* remain unanswered (Makarova *et al.* 2001). Besides features revealed by genome analysis, resistance is probably the result of a very complex combination of modifications of proteins, nucleic acids and other cell components that are not readily inferred from the sequences. The availability of several complete genomes of hyperthermophiles, including 3 different species of *Pyrococcus* (Kawarabayasi *et al.* 1998, Lecompte *et al.* 2001, Robb *et al.* 2001), opens the way for more detailed research on specific metabolic pathways which are involved in genome integrity and cell detoxification. Like *D. radiodurans*, *Pyrococcus furiosus* has an extraordinarily high capacity for repair of radiation-induced double-strand breaks (DiRuggiero *et al.* 1997). Interestingly, the same authors demonstrated that at the sequence level, only a few genes share homology with known bacterial repair genes. Phylogenetic analysis indicates that archaeal recombinases occur in two paralogous gene families, one of which is very deeply branched, and both recombinases are more closely related to the eukaryotic Rad51 and Dmcl gene families than to the *E. coli* recA gene (DiRuggiero *et al.* 1999). Comparisons of double-strand breaks induced by gamma radiation in genomic DNA of *P. furiosus* and *abyssi*, *D. radiodurans* and *E. coli* have shown that the strong

radioresistance of *Pyrococcus* is not related to a specific protection of DNA. It might partly be linked to the smaller size of the *Pyrococcus* chromosome compared to *E. coli* (1.8 Mb vs 4.6 Mb) and to the homologous recombination processes, taking advantage of the existence of several copies of their chromosomes during the log and stationary phases of growth (Gérard *et al.* 2001).

Many low molecular weight metabolites and coenzymes like ATP and NAD(P) used by archaeal hyperthermophiles have short half-lives at their optimum growth temperatures and are unstable at 110°C (Daniel & Cowan 2000). The way these microorganisms have circumvented this instability is still open to research.

## CONCLUSIONS

During the last two decades, more and more new thermophiles and hyperthermophiles from deep-sea hydrothermal vents have been discovered despite the high cost and relative difficulties in sampling. Assessing the global diversity of these extreme environments for microorganisms remains a challenging task as demonstrated by the incongruity of the cultivation and molecular retrieval approaches. A combination of both approaches is obviously needed to better characterise the microbial diversity and obtain novel species in culture. Allocating already isolated species and microorganisms evidenced only by their 16S rDNA sequences to a precise microhabitat on chimney structures has long been difficult for technical reasons. In the near future, the improvement of sampling procedures and of *in situ* monitoring of physico-chemical parameters is expected to produce data which are deeply needed to understand the ecology of hyperthermophiles. Coupling microbiology with geochemical and physical dynamics of deep-sea hydrothermal vent structure is also a promising approach. The main interest of the molecular approach was the construction of 16S rDNA libraries from which completely new taxa emerged. From sequences, it is impossible to infer the thermophily of the corresponding microorganisms and much broader temperature ranges have consequently to be tested for enrichment cultures and subsequent isolation procedures. Isolation of novel  $\epsilon$ -*Proteobacteria* like *Caminibacter hydrogenophilus* illustrates this approach.

Considerable progress has been made in recent years in the study of molecular mechanisms that enable the proteins of hyperthermophiles to operate under extreme conditions. The major determinants of protein thermostability have been characterised for many different proteins and revealed the lack of



universal law to solve the transition from mesophilic to hyperthermophilic status. Modelling the corresponding processes is consequently almost impossible and directed evolution through DNA shuffling is still the basic method to engineer proteins in order to adjust their properties to industrial requirements. The upper limit of life appears to be less constrained by protein stability, which in many cases could tolerate life up to 150°C, than by limited stability of nucleic acids and low molecular weight compounds. Moreover, most of available data were gathered from *in vitro* experiments and the shortage of data is acute for *in vivo* conditions. The complex interactions between the various cell components and the resulting possible stabilising effects make it difficult to evaluate the true stabilities of these molecules *in vivo*.

The increasing availability of complete genomes, and more specifically genomes from extremophiles isolated from deep-sea hydrothermal vents is an invaluable source of data for the near future. Despite the high percentage of ORFs with undetermined functions, averaging 50% in some cases, the evidence of interkingdom lateral gene transfer (Koonin *et al.* 1997, Nelson *et al.* 1999) and the possible resulting chimeric origin of Archaea are strong incentives for genomic research. Genome comparisons between phylogenetically close species like the 3 *Pyrococcus* have evidenced the high plasticity of these genomes (Lecompte *et al.* 2001, Myllykallio *et al.* 2000). It might also be of considerable interest in reconstructing metabolic pathways and help to design experiments for their validation.

Finally, it might be stressed that the way we see the extreme conditions prevailing at deep-sea vent fields is probably inappropriate. Microorganisms have been thriving for millions or even billions of years in these "extreme" conditions and not only survived, but developed strategies for colonising almost every type of habitats including subterranean reservoirs under the sea floor. These harsh conditions are probably the driving force to molecular adaptations which are still to be uncovered.

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