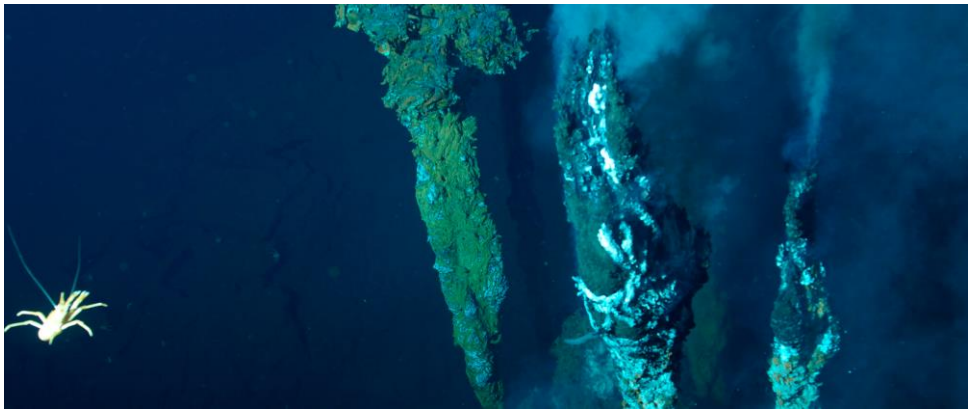
BOOK OF ABSTRACTS



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4th International Workshop on Deep Sea Microbiology (DSM)

Brest, September 15-17, 2014

During the Extremophiles Conference held in Brest in September 2006, the idea of a series of workshops dedicated to deep sea microbiology was launched. Prof. Dr. Xiao Xiang (University of Shanghai, China) took the job and organized the first edition in Xiamen (China) in November 2008, where he was settled at that time. This meeting was very successful and a second edition has been organized in 2010 in Brest, France. Again, the 3rd edition was organized by Prof Xiao Xiang in Shanghai in October 2012 and it is time to schedule the 4th edition in Brest in September 15-17, 2014.

The aim of the workshop is to gather international experts in the field of deep sea microbiology, and give them the opportunity to present very recent data, and to discuss future cooperative works, in a friendly atmosphere. Attendance and lectures are on invitation only, but the meeting is open for audience to PhD students and post-docs working at the hosting institute. This will give young scientists opportunity to listen to up to date talks, meet and discuss with experts during the breaks.

Organized by

The laboratory of Microbiology of Extreme Environments and IUEM

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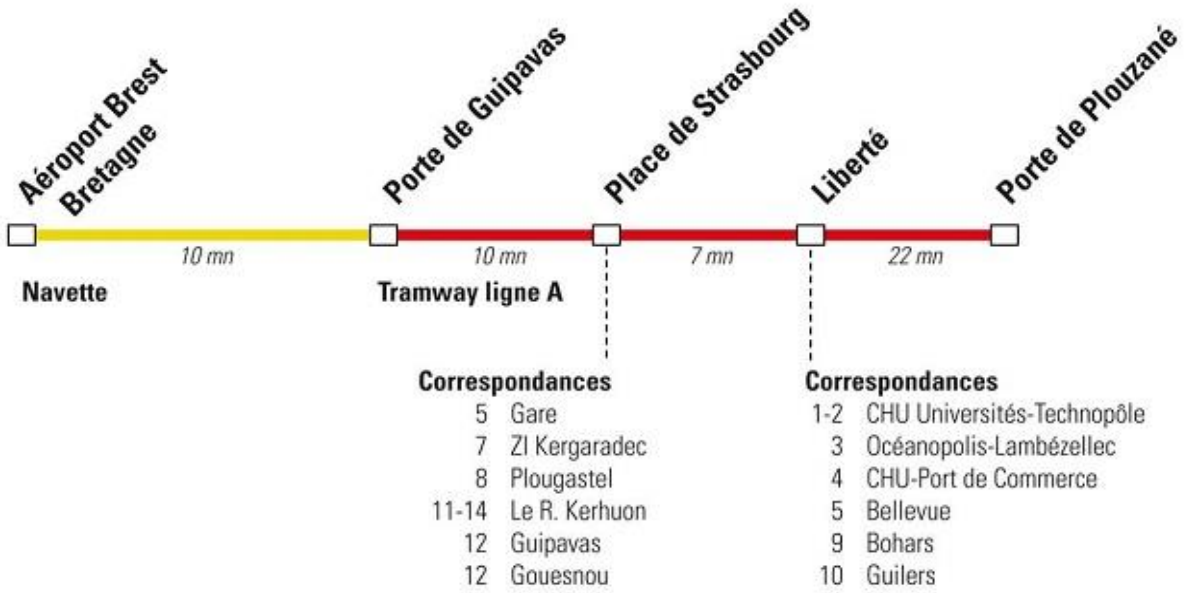
General informations

Venue : the workshop will be held at the Institut Universitaire Européen de la Mer (http://www-iuem.univ-brest.fr/en?set_language=en) IUEM, Université de Bretagne Occidentale, Technopole Brest-Iroise, Plouzané, France.

Airport: the recommended landing place is Brest-Bretagne (BES) Airport (direct flights from and to Paris CDG, Paris Orly, Lyon Saint Exupery, Marseille airport), which links Brest to the whole of Europe. It is less than one hour from Paris (10 flights per day), and only one hour from London. Other direct-flight destinations include Nantes, Lyon, Nice, Marseille, Toulon, Birmingham, Exeter and Southampton.

Airport-campus transportation: the journey by taxi from the airport to the IUEM takes 30 to 40 minutes. Several times a day, the shuttle (http://www.brest.aeroport.fr/en/access-and-parking/airport-shuttle-bus) connects the Brest Bretagne airport with the tramway station at Porte de Guipavas. Journey time: 10 minutes. Outside the tramway operating hours, the shuttle runs between the railway station and the airport.



From Place de la Liberté take the Tramway A to Porte de Plouzané and then the bus n 13 to get to the IUEM at Technopôle Brest-Iroise.

Accommodation: a single room has been booked for each invited participant from September 14 to September 17 (3 nights), in one of the following hotels all located in down town [hotels: Hotel Oceania (http://www.oceaniahotels.com/hotel-oceania-brest-centre), Hotel l'Amirauté (http://www.oceaniahotels.com/hotel-amiraute-brest) or Hotel Kyriad (http://www.kyriad-brest-centre.fr/fr)]. The accommodation will be paid by the workshop organization.

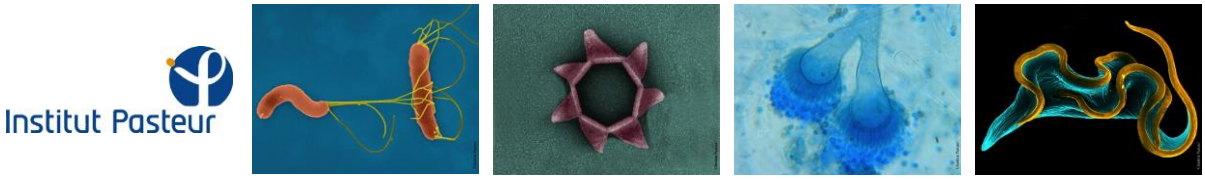
Meals: lunches on September 15 and 16 will be organized on site. Dinners on September 15 and 16 will be organized in local restaurants. All meals will be paid by the workshop organization.

Travel: as agreed, the organization will contribute to the journey costs of invited attendees

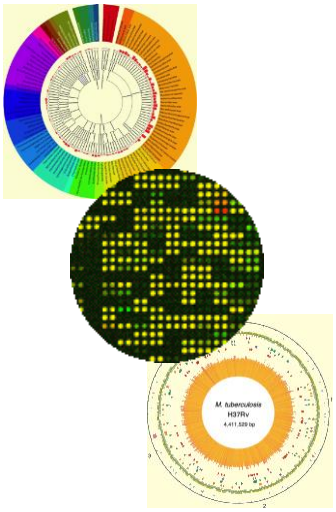
Registration desk will be open on Monday September, 15, 2014 from 8:30 am

Instruction for oral presentation: you can bring your own laptop computer or a USB flash memory if you use the equipped PC, installing PowerPoint for Windows. All speakers are requested to contact the speaker's Desk in the large conference room before the start of your session. Presenting allotted time is 30 min including discussion and change of speakers, speakers are kindly asked to keep the time of their presentations.

Special Issue of deep-sea microbiology research: selected papers presented at the conference will be published as regular papers, not as conference proceedings papers, in the journal Research in Microbiology (http://www.journals.elsevier.com/research-in-microbiology/). Manuscripts should be submitted via the Journal on-line submission system no later than November 30, 2014. Manuscripts received after this date will likely not be considered for publication in the special edition.



***Research in* Microbiology**



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Only original and well written papers exclusively reporting unpublished results of high quality and potentially substantial impact in the field will be considered for publication. Overviews of previous work, progress reports and work already published in a similar form will not be considered. All manuscripts will be screened upon submission to eliminate those that do not meet these criteria. Manuscripts that pass the pre-screen will be reviewed rigorously by at least two referees. The editors will make the final decision on the acceptability or otherwise of the manuscripts.

Monday, September 15, 2014

8:30 Registration

9:15 Welcome address (President of the university, or deputy president, director of IUEM, the Labex Mer and the organizer of the workshop)

Session 1

Session Chairs: Ken Takai and Phil Oger

10:00 Xiao Xiang (Shanghai Jiao Tong University, Shanghai, China)
A model to illustrate microbiological adaptation to the extremes p. 10

10:30 Anna Louise Reysenbach (Portland State University, USA)
Geochemical and biogeographical effects on microbial colonization of deep-sea hydrothermal deposits p. 11

11:00 Coffee break

11:30 Stefan Sievert (Woods Hole Oceanographic Institution)
Evidence for *Epsilonproteobacteria* a driver for chemosynthesis at diffuse flow deep sea hydrothermal vents p. 12

12:00 Matthieu Landreau (LM2E-IUEM-Brest)
Immobilization of marine microorganisms isolated from deep sea hydrothermal vent p. 13-14

12:30 Lunch

Session 2

Session Chair: Xiao Xiang and Peter Girguis

14:00 Ken Takai (Jamstec, Yokosuka, Japan)
Epibiotic microbial community of *Shinkaia crosnieri*: an excellent example of deep-sea chemolithotrophic microbial ecosystem interacting between environment and life p. 15

14:30 Julie Reveillaud (Marine Biological Laboratory, Woods Hole)
Subseafloor microbial communities in hydrogen-rich vent fluids from mafic and ultramafic-influenced hydrothermal sites along the Mid-Cayman Rise p. 16

15:00 Simon Le Bloa (LM2E-Ifremer-Brest)
Toward a better understanding of the symbiotic relationships in *Rimicaris exoculata* model p. 17

15:30 Coraline Mercier (LM2E-IUEM-Brest)
Discovery of bacterioviruses amongst the order of *Thermotogales* p. 18-19

16:00 Coffee break

Session 3

Session Chair: Gwenaelle Le Blay and Lois Maignien

16:30 Shao Zongze (Third Institute of Oceanography, SOA, Xiamen, China)
Diversity of chemoautotrophic sulfur-oxidizers in deep-sea hydrothermal systems and their cultivation p. 20

17:00 Karine Alain (CNRS-Brest)
Subseafloor biosphere of the Canterbury basin: diversity and physiological potential p.21-22

17:30 Gaetan Burgaud (LUBEM-ESIAB-Brest)
Astonishing fungal diversity in deep-sea marine ecosystems: an untapped resource of biotechnological potential? p. 23

18:00 Murat Eren (Marine Biological Laboratory, Woods Hole)
Information theory-based methods for microbial community analysis provide new insights on community structure and dynamics p.24

20:00 Dinner (on invitation only)

Tuesday, September 16, 2014

Session 4

Session Chairs: Anna Louise Reysenbach and Stefan Sievert

8:30 Michail Yakimov (Institute for Coastal Marine Environment, Messina Italy)
Evidence of heterotrophic bicarbonate assimilation in hadopelagic realm of Eastern Mediterranean p. 26

9:00 Sandrine Bessette (LM2E-Ifremer-Brest)
Insights into microbial communities of marine sediment from the unexplored Congo deep-sea fan lobes p. 27

9:30 Kostas Konstantinidis (Georgia Institute of Technology, Atlanta)
Metagenomic views into how life adapts to the deep sea p. 28

10:00 Peter Girguis (Harvard University, Cambridge, MA)
Eating and breathing in the deep sea: contrasting carbon cycling in energy-rich and poor deep sea environments p. 29

10:30 Coffee break

Session 5

Session Chair: Elizaveta Bonch-Osmolovskaya and Mohamed Jebbar

11:00 Elizaveta Bonch-Osmolovskaya (Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscou)
New thermophilic Bacteria from deep sea hydrothermal sources p. 30-31

11:30 Jung-Hyun Lee (Korea Institute of Ocean Science & Technology)
Comprehensive analysis of hydrogenases in the hyperthermophilic archaeon *Thermococcus onnurineus* NA1 p. 32

12:00 Tatyana Sokolova (Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscou)
Mutual effect of C1-compounds utilization processes in deep-sea *Thermococcales* p. 33

12:30 Alexander Lebedinsky (Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscou)
Genomic determinants of hydrogenogenic utilization of CO and formate in *Thermococcales* isolates from deep-sea hydrothermal vents p.34-35

13:00 Lunch

Session 6

Session Chairs: Michael Yakimov and Bruno Franzetti

14:30 Phil Oger (CNRS-Lyon)
The membrane structure of the piezophilic archaeon *Thermococcus barophilus* p.36

15:00 Long Fei Wu (Institut de Microbiologie de la Méditerranée, Marseille)
Genomic and physiological analysis of deep-sea luminous, piezophilic *Photobacterium phosphoreum* ANT2200 p. 37

15:30 Didier Flament (LM2E-Ifremer-Brest)
Protein-protein interactions network of genomic maintenance in Pyrococcus abyssi p. 38

16:00 Coffee break

16:30 Bruno Franzetti (CNRS-Grenoble)
High-pressure and functional studies of metabolic enzymes from different deep-sea microbes p. 39

17:00 Chiaki Kato (Jamstec, Yokosuka, Japan)
Pressure adaptation of the deep-sea enzymes and discovery of the high-pressure X-ray systems p. 40

17:30 General discussion

20:00 Dinner (on invitation only)

Wednesday, September 17, 2014

9:00-12:00 Tutorial

Oligotyping and Maximum Entropy Decomposition: new methods for microbial community analysis with improved ecological and biological coherence". By **A Murat Eren, Julie Reveillaud, and Lois Maignien**.

Oral Communications

Monday, September, 15, 2014

A model to illustrate microbiological adaptation to the extremes

XIAO Xiang[1,2],*, XU Jun[1,2],*, ZHANG Yu[1,2]

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Abstract:

The term "extreme environments" describes the conditions that deviate from what mesophilic cells can tolerate. By reviewing the natural history of Earth, we have a better systemic understanding of the adaptation strategies used by microorganisms in response to modern extreme environments. Genes originated approximately 3 billion years ago. After that event, life started to develop under extreme conditions, such as temperature, pressure, pH, oxidative stress, radiation, drought, heavy metal, and poison (David and Alm, 2011). All of these environmental factors influenced the formation of the metabolic pathways that are still present in the modern microorganisms.

Temperature is the core environmental factor that microorganisms must address through the adaptation process. The microbial membrane plays an essential role during adaptation because of its (im)permeability and transmembrane transportation capacity. ……In extreme environments, the reactive oxygen species generated from an imbalance in oxidation and reduction can cause damage. The poly-extreme stresses imposed by the changing of various types of other environmental parameters, could be alleviated by modulating the redox balance of the cell. (Xiao and Zhang, 2014).

To test our hypothesis, two model strains *Shewanella piezotolerans* WP3 (piezotolerant/psychrotolerant bacteria) and *Pyrococcus yayanosii* CH1 (obligate piezophilic /hyperthermophilic archaea) were chosen for cytoplasmic redox potential shift via mutation. As we expected, both mutants show different pressure/temperature/pH/[NaCl] growth range compare with the wild type strains. Our work may provide a new way to study the adaptation strategy not just qualitatively but also quantitatively.

Reference:

David L A L, Alm E J E. 2011. Rapid evolutionary innovation during an Archaean genetic expansion. Nature, 469: 93–96

Xiao X, Zhang Y. 2014. Life in extreme environments: approaches to study life-environment co-evolutionary strategies. Science China: Earth Sciences, 57: 1–6

Geochemical and biogeographical effects on microbial colonization of deep-sea hydrothermal deposits

Anna-Louise Reysenbach
Biology Department, Portland State University, Portland OR97201, USA

Over the past decade, we have gained much greater insights into the diversity of microbes at deep-sea hydrothermal vents. In particular, the diversity of microbes that rapidly colonize actively forming hydrothermal deposits as the hot hydrothermal fluid mixes with the cold oxygenated seawater, can be in part predicted based on the geochemistry of the fluid and the type of deposit. In both the bacterial and archaeal communities, using high throughput 16S rRNA gene-tagged pyrosequencing and metagenomic analysis, certain phylogenetic clades drive the observed community structural and functional differences. However, it is not geochemistry alone that influences the patterns of microbial diversity at vents. Multi-locus sequencing data from several different deep-sea hydrothermal genera such as *Persephonella* and *Aciduloprofudum*, confirm that in addition the geochemical forces affecting hydrothermal vent microbial communities, at the genomic and genus level, vent microbes exhibit a biogeography.

Evidence for *Epsilonproteobacteria* a driver for chemosynthesis at diffuse flow deep sea hydrothermal vents

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Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

Deep-sea hydrothermal vents are exemplary ecosystems where microbial chemosynthesis rather than photosynthesis is the primary source of organic carbon. Chemosynthetic microorganisms are at the nexus of these systems by effectively transferring the energy from geothermal sources to higher trophic levels. While the validity of this conceptual framework is well established, there are still major gaps in our understanding of the microbiology and biogeochemistry of deep-sea hydrothermal systems. Important questions in this regard are: (1) How much, at what rates, and where in the system is organic carbon being produced? (2) What are the dominant autotrophs, where do they reside, and what is the relative importance of free-swimming, biofilm-forming, and symbiotic microbes? (3) Which metabolic pathways are they using to conserve energy and to fix carbon? (4) How does community-wide gene expression in fluid and biofilm communities compare? and (5) How efficiently is the energy being utilized, transformed into biomass, and transferred to higher trophic levels? In particular, there is currently a notable lack of process-oriented studies that would allow an assessment of the larger role of these ecosystems in global biogeochemical cycles. To this end, we are pursuing integrated biological and geochemical studies to gain insights into the underlying processes and the organisms catalyzing them. In particular, meta-genomic and -proteomic studies combined with activity measurements are helping us to not only identify the predominant microorganisms, but also to reveal the expressed metabolic pathways, and thus main energy sources driving chemoautotrophic production, as well to identify metabolically active cells by performing incubations under simulated in situ conditions. Our analyses are suggestive of functional redundancy within the community, with a core set of metabolic repertoire despite a high microdiversity as indicated by 16S rRNA based surveys. Thus, the high taxonomic diversity observed at deep-sea vents is not necessarily reflected in functional diversity, such that different taxa can perform similar functions using homologous pathways, but being optimally adapted to slightly different environmental conditions. Such a strategy might impart robustness to the overall performance of the community and its response to the dynamic vent environment that is characterized by steep thermal and redox gradients, as well as frequent disturbances.

Immobilization of marine microorganisms isolated from deep sea hydrothermal vent

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[2]LISA/METICA, Faculté des Sciences, Avenue escadrille Normandie Niemen 13397 Marseille
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Marine microorganisms, including those living in extreme environments such as deep-sea hydrothermal vents play a crucial role in Earth biogeochemical cycles. However, despite the significant progress made on the knowledge of the biodiversity of these microorganisms, their culture remains problematic. Lack of medium renewal, culture conditions fare from environmental conditions and the lack of interactions between microorganisms are often mentioned to explain these difficulties [1]. Cultivation of microbial communities immobilized in a polymer matrix appears as a good way to mitigate some of these limitations, as it has been shown for other microbial systems [2]. This technic should enable to maintain a community of thermophilic microorganisms during long term continuous culture in a gas-lift bioreactor [3], to avoid the leaching of less competitive species and to improve cell-cell interactions. The objectives of this study are (i) to develop a protocol suitable for the immobilization of thermophilic and hyperthermophilic marine microorganisms, (ii) test the use of polymers in a range of physical and chemical conditions and (iii) validate the method with a continuous culture of a synthetic immobilized community. The best conditions for immobilization were obtained with a mixture of gellan (2.5%) and xanthan (0.25%) gums with a salt concentration of 12 g / L, an emulsion temperature of 80 °C and a stirring speed of 250 rpm / min. Beads resistance in different culture conditions (temperature, pH, sulfur and salt concentrations) was also tested. After 5 weeks of incubation, beads showed a good resistance for a pH between 5.4 and 8, a maximal temperature of 90 °C and no significant effect of salt and sulfur concentrations tested on beads diameter.

In order to confirm the method efficiency, a continuous culture in a gas-lift bioreactor was performed with an immobilized mixture of autotrophic and heterotrophic deep-sea hydrothermal microorganisms. First results showed efficient growth of different species of microorganisms (concentration average up to 8.3 ± 0.1 Log cell/ml of effluent) even after a long period (7 days) of stress (oxygen, pH). Further analyses will provide more informations about the biodiversity present in the bioreactor throughout the culture.

Keywords : Immobilization, Gas-lift bioreactor, Gel bead, Continuous culture, Microbial ecology.

References :

[1] POSTEC A, LESONGEUR F, PIGNET P, OLLIVIER B, QUERELLOU J, GODFROY A (2007) : Continuous enrichment cultures: insights into prokaryotic diversity and metabolic interactions in deep-sea vent chimneys. Extremophiles, 11(6): 747-757.

[2] ALAIN K, QUERELLOU J (2009) : Cultivating the uncultured: limits, advances and future challenges. Extremophiles, 13(4): 583-594.

[3] LE BLAY G, CHASSARD C, BALTZER S, LACROIX C (2010) : Set up of a new in vitro model to study dietary fructans fermentation in formula-fed babies. British Journal of Nutrition, 103(3): 403-411.

Epibiotic microbial community of *Shinkaia crosnieri*: an excellent example of deep-sea chemolithotrophic microbial ecosystem interacting between environment and life

Ken Takai and Tomoo Watsuji, Department of Subsurface Geobiological Analysis and Research (D-SUGAR), Japan Agency for Marine-Earth Science & Technology (JAMSTEC)

Hydrothermal vent crab *Shinkaia crosnieri* is one of the most dominant chemosynthetic animal species in the deep-sea hydrothermal systems of the Okinawa Trough. It has a dense and unique epibiotic chemolithotrophic microbial community mainly composed of epsilon-proteobacterial and gamma-proteobacterial thiotrophs, additionally in some highly methane-enriched fields, gamma-proteobacterial metahnotrophs, in the dorsal setae. A combination of 13C-tracer experiments, microscopic fluorescence in situ hybridization (FISH) and nano-scale secondary ion mass spectrometry (nano-SIMS) have also indicated that the predominant *Sulfurovum*–affiliated epibiotic population represents primary thioautotrophic productivity in the epibiotic community. In addition, a combination of 13C-tracer experiment and transcriptomic analysis has clarified that the gamma-proteobacterial type Ia and Ib methanotrophs in *S. crosnieri* epibiotic community also support primary production using CH4 as the energy and carbon sources and has been considered nutritionally to be connected with its epibiotic bacteria while the nutrition transfer manner has remained equivocal. Since the first discovery of *S. crosnieri* in the Okinawa Trough hydrothermal systems, it has been observed that living *S. crosnieri* individuals frequently show a feeding-like behavior, for example, they comb out the setae covered with the dense epibionts using the third maxilliped and then brought the maxilliped to the mouth. However, none of the clear evidences has been yet obtained to prove this hypothesis. Recently, we have succeeded in obtaining the clear evidences. Phylogenetic analysis revealed that most of bacterial 16S rRNA gene sequences in the intestine were related to the epibionts' sequences. Observation of intestinal microbial assemblages after a dye-stained-epibiont-tracer experiment using living *S. crosnieri* also found the being-digested epibiont materials as well as the setae fragments. Activity measurements and isotopic characterizations indicated that the gut microbial components were metabolically inactive and 13C-carbon assimilated in the epibionts served as carbon (nutrition) source of the host. Combined with feeding-like behavior of living *S. crosnieri*, these results indicate that *S. crosnieri* ingests the epibionts using maxillipeds, and assimilate them in the intestine as nutrition source. This is a historical example that the nutritional transfer manner in the ectosymbiosis between chemosynthetic bacteria and deep-sea invertebrates is completely clarified.

Subseafloor microbial communities in hydrogen-rich vent fluids from mafic and ultramafic-influenced hydrothermal sites along the Mid-Cayman Rise

Julie Reveillaud[a], Emily Reddington[a], Jill McDermott[b], Julie L. Meyer[c], Sean Sylva[b], Jeffrey Seewald[b], Christopher R. German[b], and Julie A. Huber[a]

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Abstract

Warm fluids emanating from hydrothermal vents are one of the best windows into the rocky subseafloor habitat and its resident microbial community. Here, we determined the abundance, community composition, diversity and chemical environment of bacteria and archaea in venting fluids from two newly discovered deep-sea hydrothermal systems along the ultra-slow spreading Mid-Cayman Rise (MCR) using a combination of chemical measurements, total cell and domain-specific enumeration, targeted stable isotope tracing experiments, cultivation enrichments, and 16S rRNA gene amplicon sequencing. The mafic-hosted Piccard at 4960 meters and ultramafic-influenced Von Damm at 2350 meters, each represent novel geologic settings for deep-sea hydrothermal vents with distinctive fluid chemistry. Despite large differences in depth, geologic setting, and vent fluid chemistry, the sites are located only 20 km apart and both host fluids highly enriched in hydrogen. Results indicate the presence of vent endemic microbial communities at each site, with communities at one vent field being more similar to neighboring orifices than to orifices at the other vent field. In addition, intra-field microbial community variation was strikingly reduced at Piccard while more variation was observed at Von Damm, where overall community richness was higher than at Piccard. Methanogenic activity with formate was only observed at Von Damm, while enrichment of thermophilic heterotrophs was only successful at Piccard. However, different lineages within the hydrogen-utilizing genera *Methanothermococcus*, *Archaeoglobus*, and *Sulfurovum* were predominant at both sites, consistent with the key role hydrogen plays in driving microbial community structure at deep-sea hydrothermal vents.

Toward a better understanding of the symbiotic relationships in *Rimicaris exoculata* model

Simon Le Bloa, Lucile Durand, Laure Taupin, Charlotte Marteau, Marie-Anne Cambon-Bonavita, Alexis Bazire.

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In the deep oceanic hydrothermal vents and cold seeps, light does not penetrate. Chemolithoautotrophic micro-organisms are the main primary producers, using reduced compounds from the fluids or the minerals. In such extreme environments, bacterial symbiosis frequently occurs. This is the case of the caridean shrimp *Rimicaris exoculata*. This is an endemic species of the Mid-Atlantic Ridge hydrothermal vent sites, which dominates the macrofauna of many of them. A dense ectosymbiotic bacterial community, associated with mineral oxide deposits, colonises its enlarged gill chamber. Its structure and establishment process reminds that of biofilm ones which use a communication pathway dependent on the bacterial density called Quorum Sensing (QS).

Up to date the recognition system and cell-cell communication between host and symbionts and between symbionts themselves is unknown, while it must be effective as the symbiotic populations are stable in time (life cycle) and in space (regardless of the hydrothermal site origin). The presence and activity of genes involved in QS were investigated by PCR, RT-PCR and Q-PCR.

The presence of the *luxS* gene in the epibiontic community of *R. exoculata* at different moult stages was confirmed for the Rainbow and TAG vent sites. The RT-PCR experiments were able to highlight a potential activity of QS only for shrimps of the TAG and Rainbow vent sites. Phylogenetic analysis affiliated the *luxS* gene to *Sulfurovum* sp. NBC37-1 for the Rainbow vent site and to *Arcobacter* sp. for the TAG one. Preliminary Q-PCR experiments suggested a correlation between the expression of luxS gene and the development of the epibiont affiliated to the *Epsilonproteobacteria*. The characterization and quantification of communication molecules (acylhomoserine lactones (AHLs)) have been done by LC-MS-MS.

Keywords: symbiosis, *Rimicaris exoculata*, molecular biology, biochemistry, interactions, bioactive molecules.

Discovery of bacterioviruses amongst the order of *Thermotogales*

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Our knowledge of the viral diversity associated to the procaryotic microorganisms inhabiting the deep sea hydrothermal vents is still limited. Only few studies have focused on viral abundance and impact on microbial mortality within these ecosystems. A limited number of viruses from these environments were isolated and characterized. Two viruses associated to hyperthermophilic anaerobic *Archaea*, *Thermococcales*, have been described in our laboratory (1, 2). In order to deepen our knowledge on the viral diversity of marine hydrothermal microorganisms, we have extended our investigation to the bacterial order of *Thermotogales*. This order is composed of anaerobic chemoorganotrophic bacteria that are, for almost, hyper/thermophilic. They share the same ecological niche with the *Thermococcales*. Numerous lateral gene transfers have contributed to the evolutionary history of the *Thermotogales*, implying the potential involvement of viruses (3). However, up till now, only two miniplasmids as mobile genetic elements have been described within *Thermotogales* (4).

Sixty strains of *Thermotogales* were screened for mobile genetic elements. Extrachromosomal DNA elements, including bacterioviruses belonging to the Siphoviridae, were discovered. One new virus-host system was characterized in details, MPV1 (*Marinitoga piezophila* virus 1), (Lossouarn J. and al., submitted) is a temperate Siphovirus-like isolated from a piezophilic bacterium. MPV1 is the first reported bacteriovirus that infects *Thermotogales*. MPV1 shares its host with a circular extrachromosomal genetic element of 13.3 kb (pMP1). This 'ménage à trois' is surprising in the sense where the 13 kb element, that contains 13 ORFs of mostly unknown function, uses the viral capsid to propagate. Therefore, it would likely correspond to a new example of molecular piracy. During the screening of *Thermotogales* strains, two other new system hosts/viruses were found and their characterizations are in progress. Comparative studies of the different extrachromosomal elements (viruses, plasmids and membrane vesicles), isolated from hyper/thermophilic *Archaea* and *Bacteria*, should further enhance our knowledge on their diversity, but also on their impact on their hosts and shed light on these extreme marine ecosystems.

Deep-sea vent/ Bacteria/ thermophile/ molecular piracy/ plasmid/virus

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Diversity of chemoautotrophic sulfur-oxidizers in deep-sea hydrothermal systems and their cultivation

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The microbial oxidation of reduced sulfur compounds through chemolithoautrophic processes has been expected to be the primary energy metabolism driving deep-sea vent ecosystems. More and more studies indicate that free-living and symbionts epsilon- and gamma- proteobacteria are important sulfur-oxidizers in the global deep-sea hydrothermal environments. However, the ecological and biochemical roles of these chemoautotrophs in deep sea hydrothermal vent ecosystem are still far to be unknown. This is mostly due to the lack of pure cultures of the dominant sulfur oxidizers from deep-sea vents. In this study, we focused on the diversity and physiology of these sulfur-oxidizing bacteria by cultivation-independent (metagenomic, high throughput 16S rRNA gene and function gene analysis) and cultivation-dependent techniques. The deep sea samples included hydrothermal vent sulfides, shrimps and hydrothermal fluids from the Southwest Indian Ocean and South Atlantic Mid-ridge. We found that (1) the uncultured gamma group SUP05 were the major members in the microbial community from the hydrothermal plumes, accounted for 21%-42%, while not or quite less detected in hydrothermal sulfides and shrimps. (2) Uncultured *Epsilion-proteobacteria* were found as the predominant sulfur-oxidizing endosymbionts in the hydrothermal shrimps, instead of *Gamma-proteobacteria*. (3) Some sulfur-oxidizing bacteria have been obtained after enrichment under microaerobic and anaerobic conditions. These isolates mainly belonged to the genus *Thiomicrospira*, *Arcobacter*, *Thalassospira*, *Marinobacter* and *Pseudomonas*. *Gamma-proteobacteria* belonging to genus Thiomicrospira were found predominant in the sulfur-oxidizing communities derived from the deep-sea hydrothermal vents. Bacteria of genus *Arcobacter* of *Epsilion-proteobacteria* have been found dominated in the filamentous sulphur mat in hydrothermal vent system. This is the first report about the cultivation in lab of these sulphur-oxidizers from the hydrothermal vent fields.

Subseafloor biosphere of the Canterbury basin: diversity and physiological potential

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The subseafloor microbiota is diverse and complex, hosting metabolically active communities down to depths of more than 1000 meters below the seafloor (mbsf). It harbors representatives from the three domains of life, i.e., numerous endemic and/or as yet uncultured Archaea and Bacteria, in addition to bacterial endospores, protists and fungi belonging to Eukarya. Although background molecular data on bacterial and archaeal lineages inhabiting subsurface sediment above 1000 mbsf exists, most deep-subsurface microorganisms detected so far were refractory to cultivation. So far, active prokaryotes have been discovered down to 1626 mbsf, and microeukaryotes down to 113 mbsf, but the lower limit of the deep subsurface biosphere remains elusive.

Besides impacting the planetary geochemical cycles, the subseafloor biosphere and its functioning are still poorly understood. Questions related to possible metabolic pathways and, to a broader extent, to adaptive strategies that may be deployed by buried microorganisms (sporulation, response to stress, dormancy…) have been poorly investigated. In this study, we investigated the subsurface microbial communities from a core of a record-length (1927m – ~35.2-36 million years at the bottom of the core) collected in the Canterbury basin, off the coast of New Zealand, during the IODP Leg 317 expedition. A very stringent high-throughput 454-pyrosequencing approach targeting the 16S/18S rRNA genes for *Bacteria*, *Archaea* and *Eukarya*, along with real-time PCR analysis (genetic markers and functional genes), cell counts and cultures, were performed to assess their abundance, diversity and activity at different depths. We also analyzed 2 metagenomes from sedimentary layers at 31 mbsf and 136 mbsf to better understand the physiological potential and the possible lifestyles of subseafloor microbial communities.

Our results suggest also that diverse microorganisms persist down to 1922 mbsf in the seafloor of the Canterbury Basin and extend the previously known depth limits of microbial evidence (i) from 159 to 1740 mbsf for Eukarya and (ii) from 518 to 1922 mbsf for Bacteria. Shifts in microbial community composition along this very long core reflect vertical taxa zonation influenced by sediment depth. Representatives of some microbial taxa were also cultivated using methods mimicking in situ conditions. Predicted genes related to metabolism were involved in anaerobic processes such as fermentation, methanogenesis and utilization of fatty acids or aromatic and halogenated substrates, at 31 and 136 mbsf.

Potential for microbial processes that may confer selective advantages for the subsurface microorganisms were predicted, including sporulation, detoxication equipment, environmental sensing, osmolyte accumulation or DNA repair. These results suggest also that a fraction of the diversity might be dormant. Manual annotation of large contigs described the metabolic versatility of MCG, Chloroflexi and Euryarchaeota and also showed that recombination events are frequent within subsurface taxa.

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Astonishing fungal diversity in deep-sea marine ecosystems: an untapped resource of biotechnological potential?

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Marine fungi have usually been considered as exotic microorganisms only fascinating a scarce panel of scientists. Ecologically important relationships between marine fungi from open oceans or coastal waters and other organisms have been clearly demonstrated. However, the diversity, ecological role(s) and biotechnological potential of fungal communities in deep-sea marine extreme environments as hydrothermal vents or deep subsurface sediments are far from being resolved. From recent surveys, hydrothermal vents and deep sediments appeared as life oases for fungi with the description of unexpected communities revealed by culture-independent and culture-based methods but also with new described species with specific adaptations to deep-sea conditions. As natural product chemolibraries from marine fungi from coastal waters are rapidly expanding, we can hypothesize that a deep exploration of fungi from extreme environments, particularly deep-sea hydrothermal vents and deep sediments appeared as an untapped reservoir of biomolecules with tremendous biotechnological potential.

As nearly 10% of genomes are devoted to secondary metabolism, genome mining to find natural product biosynthetic pathways can be seen as a new paradigm for natural product discovery. PCR targeting PKS (Polyketide Synthase), NRPS (Non-Ribosomal Peptide Synthetase), hybrids (PKS-NRPS) and TPS (Terpene Synthase) genes have been performed on 200 fungal strains isolated from deep-sea ecosystems. Around 97% of deep-sea fungal strains harbor at least one of those genes revealing the biotechnological potential of this new collection. Finally, fungal strains are currently screened for their antimicrobial activities against 10 clinical pathogens. On 140 strains screened, 18% show activities against E. coli, S.aureus and E. faecalis. The most promising strains will be selected and their metabolite production will be studied. Molecules with potential applications will be isolated and identified using LC-MS/MS and NMR.

Information theory-based methods for microbial community analysis provide new insights on community structure and dynamics.

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Molecular microbial ecology investigations often employ large marker gene datasets, e.g., ribosomal RNAs, to represent the occurrence of single-cell genomes in microbial communities. Massively parallel DNA sequencing technologies now enable extensive surveys of marker gene libraries. Community similarity assessment traditionally involves computational approaches relying on pairwise sequence alignments and clustering with de facto similarity thresholds to mitigate influence of sequencing errors (e.g. 97% OTU clustering). Such approaches, however, have prevented fine-scale resolution descriptions of microbial communities.

Minimum Entropy Decomposition (MED) provides a computationally efficient means to partition marker gene datasets into "MED nodes", which represent homogeneous, ecologically meaningful operational taxonomic units. By employing Shannon entropy, MED only uses the information-rich nucleotide positions across reads and iteratively partitions large datasets while omitting stochastic variations. MED can identify organisms that differ by as few as 1 nt over the sequenced region as distinct units regardless of the sequence length and percent similarity. The information theory-guided decomposition process behind the MED algorithm enables sensitive discrimination of closely related organisms in marker gene amplicon datasets without relying on extensive computational heuristics and user supervision. Using recent example from sponge/sewer/mouth microbiome, I will show how accessing the distribution of closely related microorganisms can broaden our understanding of microbial community structure and functioning, down to the ecotype level. Such approaches could find useful applications in environments with comparatively little knowledge on taxonomic nomenclature and classification, such as remote and extreme deep-sea environments.

Oral Communications

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Evidence of heterotrophic bicarbonate assimilation in hadopelagic realm of Eastern Mediterranean.

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Background: Active bicarbonate fixation in deep dark ocean was recently postulated be mainly attributed to the ammonium-oxidizing chemoautotrophic organisms belonging to Marine Group I of Thaumarchaeota. But in some studies, deviation in thaumarchaeal abundance and measured rates of dark primary production was described. Hence, dark primary production in the biggest ecosystem on our planet may be relevant for a wide range of organisms not necessarily belonging exclusively to chemoautotrophs. To elucidate this, we decided to use comprehensive analysis approach to study the mediators of dark bicarbonate assimilation pointing out to the importance of anaplerotic reactions driven by heterotrophic bacteria. Previously characterized water column at Matapan-Vavilov Deep Station (MVD) (Eastern Mediterranean) was chosen as a model bathypelagic ecosystem.

Results: Measurement of [14C]-bicarbonate uptake rates revealed a discrepancy between archaeal composition and fixation activities. 14C-bicarbonate incorporation experiments combined with shotgun [14C]-proteomic analysis identified at least 95 different proteins of bacterial origin, among which more than quarter was attributed to *Alteromonas macleodii* 'deep ecotype' AltDE1, a dominated organism in MVD bathypelagic microbial community. This organism remained also dominant in incubation tests. Cultivation experiments confirmed high A. macleodii AltDE1 capability of bicarbonate uptake. During 96 hours of cultivation at 14°C in the dark and at ambient concentrations of both dissolved organic and inorganic carbon, the daily cellular rates of bicarbonate fixation by A. macleodii AltDE1 were between 1.66 ± 0.07 (n=3) and 2.08 ± 0.15 (n=3) fg C cell-1 day-1.

Conclusions: Dark primary production in bathypelagic interior of Mediterranean Sea is associated with different metabolic processes, including chemolithoautotrophy and heterotrophic assimilation of bicarbonate. In the absence of active thaumarchaeotal population, the last process may significantly contribute to de novo synthesis of organic carbon in deep-sea and thus, implies an additional important role of heterotrophic bacterial populations in global biogeochemical cycles running in ocean's interior.

“Insights into microbial communities of marine sediment from the unexplored Congo deep-sea fan lobes”

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The Congo deep-sea fan lobes, situated in the Congo-Angola margin (West Africa), represent a unique deep-sea sedimentary ecosystem characterized by rapid turbidities deposition where diffuse seepages are probably originated from the recycling of rich organic carbon inputs in sediments that sustain an entire ecosystem. Furthermore, sediments rich in organic matter are likely dominated by both early diagenesis in the first meters and diverse microbial communities including methanogens, methanotrophs and sulfate-reducing bacteria. So far, the microbial communities of the deep-sea Congo lobes ecosystem are still unknown. In order to explore the bacterial and archaeal diversity, 86 marine sediment samples from four geographical locations were investigated using Illumina paired-end tag sequencing on the V6 region of the 16s rRNA gene. The high sequencing coverage allows the assessments of ecological questions related to the diversity, community composition, distribution of microbes as well as potentially reveal interactions between microorganisms and aspects of environmental patterns. This presentation would provide highlights in the understanding of the microbial diversity potentially involved in the early diagenetic processes and the generation of enriched sulfide and methane fluids in the unexplored deep-sea fan of Congo Canyon.

Metagenomic views into how life adapts to the deep sea

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Culture-independent genomic surveys (a.k.a. metagenomics) have recently provided new insights into the structure, function, and adaptations of deep-sea planktonic microbial communities. For instance, our own previous study (Konstantinidis et al., AEM, 2009) has revealed that communities are well-structured (stratified) at different depths, and a collection of very subtle changes in gene content and structure underlie the adaptation to the deep and cold biosphere. However, these previous studies were based on a limited number of samples; hence, the universality of the deep-sea signature obtained remains to be evaluated. To this end, we characterized multiple stations, over time, in the Gulf of Mexico, which included surface, mixed layer, chlorophyll maxima, oxygen minima, and ~2,000m-deep water samples. Comparisons of the first 20 Illumina shotgun metagenomes originating from these samples revealed significant shifts in taxa and functional gene content between the surface and deep communities, some of which confirmed previously reported differences while others represented previously unidentified adaptations. For instance, besides various light-driven metabolic pathways, phosphate metabolism and viral proteins were enriched in the surface, while organic sulfur assimilation and aromatic compound metabolism were enriched in the deep. The implications of these findings for better understanding deep-sea life will be discussed.

Eating and breathing in the deep sea: contrasting carbon cycling in energy-rich and poor deep sea environments.

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The last thirty-five years have been a watershed for deep-sea microbiology. The discovery of hydrothermal vents and their extraordinarily productive communities, along with the discovery of the deep subsurface biosphere and their slow-growing, energy-starved microbial communities have changed our ideas about the nature and extent of microbial life in the deep sea.

While we often compare the biomass density at vents to that of rainforests, and the nutrient limitation in the deep subsurface to that of deserts and Antarctic dry valleys, we know far more about rainforests and deserts than we do about microbial life in the deep sea. Even after decades of research, there remain a number of long-standing questions regarding the distribution and activity of microbes in situ. We know that hydrothermal vents are energy-rich environments, and the energy for microbial primary productivity at hydrothermal vents is primarily derived from compounds that are in disequilibria between hot, reduced thermal fluids and the ambient, oxidized bottom seawater. However, we have a rudimentary understanding of how microbes are distributed within this geochemical gradient, and how temporal variability in fluid flow and even eruptions influences primary and secondary productivity. Accordingly we have recently developed technologies and methods to allow us to synoptically measure geochemistry and microbial processes (community composition and gene expression) over space and time, and our findings reveal that striking patterns of microbial distribution, gene expression and activity within a vent field that challenge our conventions.

At the other extreme, deep subsurface environs can be very limiting, and microbes are likely limited in their access to either electron donors (e.g. dissolved organic matter, or DOM) or electron acceptors (e.g. oxygen). There are some deep subsurface habitats, however, where hydrological circulation brings fresh reductants and/or oxidants into the deep subsurface. We have been studying such sites to better understand carbon cycling in the deep subsurface, in particular the fate of more recalcitrant carbon in environments that are replete in oxygen (the recalcitrance of organic matter can be a function of oxidant availability). Using advanced mass spectrometry and sampling techniques, we have characterized DOM in a number of subsurface environments, identifying which compounds are subject to microbial degradation and which are recalcitrant (some compounds that have remained unaltered for over 20,000 years).

In concert, molecular microbiological and biogeochemical techniques have begun to shed some light on the relationship between microbial ecology and physiology and biogeochemical cycles, and ongoing developments promise to further bridge this gap in our knowledge about the role that microbes play in governing our deep sea biogeochemistry and, ultimately, our biosphere.

New thermophilic Bacteria from deep sea hydrothermal sources

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Significant achievements describing microbial diversity in deep-sea hydrothermal habitats have been made during past decade by means of molecular approach. However, many microorganisms remain uncultured, and, thus their role in hydrothermal communities unidentified. Here we present the results of the isolation and characterization of novel thermophilic prokaryotes from different deep-sea hydrothermal locations.

Thermophilic iron-reducing bacteria and archaea inhabiting deep-sea vents are phylogenetically diverse. A new moderately thermophilic, anaerobic, dissimilatory iron(III)-reducing bacterium *Deferrisoma camini* gen. nov., sp.nov. was isolated from a deep-sea hydrothermal vent chimney located on the Eastern Lau Spreading Centre in the Pacific Ocean at a depth of about 2150 m (1). Cells of new organism are Gram-negative ovals to short rods with a single polar flagellum. New isolate uses acetate, fumarate, malate, maleinate, succinate, propanol, palmitate, stearate, peptone and yeast extract as electron donors for growth and iron(III) reduction. All substrates were oxidized completely to CO2 and H2O. Iron(III) (in the form of ferrihydrite, ferric citrate or ferric nitrilotriacetate) and elemental sulfur (S0) were the electron acceptors that supported growth.

Results of 16S rRNA gene sequence analysis showed that the novel bacterium is related to representatives of the orders *Desulfuromonadales* and *Syntrophobacterales* with 84–86% sequence similarity and forms a distinct phylogenetic branch in the *Deltaproteobacteria*. Sulfur disproportionation is known as a catabolic process in several mesophilic bacteria, but has never been found in thermophiles. From a deep-sea hydrothermal vent chimney located on the Eastern Lau Spreading Center, Pacific Ocean, at a depth of 1910 and 2150 m two strains of sulfur-disproportionating bacteria were isolated (2, 3).

Thermosulfurimonas dismutans gen. nov., sp. nov., is an anaerobic, chemolithoautotrophic bacterium with oval or short rod-shape cells motile with a single polar flagellum, growing in the temperature range from 50 to 92oC, with an optimum at 74oC. *Dissulfuribacter thermophilus* gen. nov., sp. nov., also an anaerobic chemolithoattroph, has similar morphology but grows at lower temperatures, 28–70°C, with an optimum at 61°C. Both isolates grow by disproprotionation of elemental sulfur, thiosulfate and sulfite, with bicarbonate/CO2 as a carbon source. Analysis of the 16S rRNA gene sequence revealed that the first isolate represents a novel genus in the phylum *Thermodesulfobacteria*, while the second one forms a distinct phylogenetic branch within the *Deltaproteobacteria*.

Two new species, *Thermosipho affectus* sp. nov., and *Thermosipho activus* sp. nov., were isolated from the deep sea vents of Mid-Atlantic Ridge and Guaymas Basin, respectively (4, 5). Both isolates are able to grow on starch, cellulose and cellulose derivatives; *T. activus* utilizes also chitin, xylan, pectin and proteins including β-keratins, casein, gelatin.

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Comprehensive analysis of hydrogenases in the hyperthermophilic archaeon *Thermococcus* onnurineus NA1

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Thermococcus onnurineus NA1 was isolated from a deep-sea hydrothermal vent region to understand the adaptation mechanism of microorganisms in the highly variable hydrothermal vent environment. Based on its genome sequencing (Lee et al. 2008), genes were identified coding for a CO dehydrogenase (Codh), three formate dehydrogenases (Fdh) and unprecedented high copies of hydrogenases. The polyphasic approach by employing transcriptomic, proteomic and metabolomic tools allowed us to understand the functionality of each hydrogenase (Kim et al. 2010; Moon YJ et al. 2012; Kim et al. 2013). In this study, functionality of the soluble hydrogenase in *T. onnurinues* NA1 which shows similarity to F_{420}-reducing hydrogenase will be discussed. The F_{420}-reducing hydrogenase has been known as a key enzyme in methanogenesis. Its homologs have been identified in non-methanogenic hyperthermophilic archaea, including *T. onnurineus* NA1, but neither physiological function nor biochemical properties has been reported to date. The enzyme of *T. onnurineus* NA1 was distinguished from those of other methanogens and the members of the family *Desulfurobacteriaceae* with respect to the phylogenetic distribution of the α and β subunits, organization of *frhAGB* genes and conservation of F_{420}-coordinating residues. The trimeric enzyme complex was purified to homogeneity via affinity chromatography from *T. onnurineus* NA1 and the biochemical properties of the purified enzyme will be discussed.

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Mutual effect of C1-compounds utilization processes in deep-sea *Thermococcales*

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Formate and/or CO present in deep-sea hydrothermal fluids may in part fuel the microbial communities inhabiting deep sea hot vents (1, 2). We demonstrated the capacity for hydrogenogenic or sulfidogenic (sulfur-reducing) growth on CO or formate for thermococci isolated on different substrates from geographically distant deep sea thermal fields.
The capacities for these processes occur in thermococci in various combinations. We found that the presence of one of these substrates influences the utilization of the other one. The presence/absence of elemental sulfur as an electron acceptor also affects the utilization of CO or formate as electron donors.
For example, of four cultures capable of growth at the expense of hydrogenogenic formate utilization, only *T.gammatolerans* was able to grow on formate with sulfur. Our study of the location in the genomes of binding sites of the *Thermococcales*-specific SurR regulatror of hydrogen and sulfur metabolism (3,4) allowed us to conclude that, in thermococci capable of hydrogenogenesis but not of sulfidogenesis on formate, sulfur switches off the entire operon that includes formate transporter.
Other aspects of the regulation of the discussed processes in thermococci will also be considered, both those understandable from genome examination and those whose mechanisms yet remain unclear and need further studies.
Study of the regulation of the discussed processes and its genomic mechanisms may shed light on their ecological role.

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Genomic determinants of hydrogenogenic utilization of CO and formate in *Thermococcales* isolates from deep-sea hydrothermal vents

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Data on CO and formate concentrations in hydrothermal fluids are so far scarce. However, CO concentration of 5 µM has been reported for fluids of the Rainbow vent site on the Mid-Atlantic Ridge (Charlou et al. 2002) and formate concentrations as high as 36-158 µM have been recorded in fluids of the Lost City hydrothermal field (Lang et al. 2010). We have isolated several strains of *Thermococcus* species from various deep-sea hydrothermal sites under selective conditions favoring hydrogenogenic growth on CO or formate (Sokolova et al. 2004, Kim et al. 2010, and our unpublished data). Their genomes were sequenced and specific gene clusters were found that determine CO- or HCOOH-driven hydrogenogenic growth. Four of these isolates proved to be phylogenetically close to *Thermococcus barophilus*. The strains of this group, as well as the *T. barophilus* type strain MP (Marteinsson et al. 1999; Vannier et al. 2011), share several highly similar hydrogenase gene clusters, one of which is adjoined by a carbon monoxide dehydrogenase gene and determines the capacity for hydrogenogenic growth on CO. Two of these new isolates additionally have three more hydrogenase gene clusters, of which one, adjoined by formate dehydrogenase and formate transporter genes, determines the capacity for hydrogenogenic utilization of formate. These differences within the phylogenetically coherent group raise questions as to whether they are consequences of horizontal gene transfer or differential gene loss.

Notably, several *Thermococcales* strains isolated organotrophically contain gene clusters determining the capacity for CO- and/or HCOOH-driven hydrogenogenic growth, and for some of them these capacities were tested and indeed demonstrated (Lee et al. 2008, Kim et al. 2010, and our unpublished data).

Thus, hydrogenogenic utilization of CO and formate seem to be ecologically relevant types of metabolism. Study of the phylogeny of the genomic determinants of these processes is to shed light on their possible ancient nature and ways of their inheritance.

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The membrane structure of the piezophilic archaeon *Thermococcus barophilus*

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Introduction. As a barrier between the cell and the environment biological membranes play an central role. They are highly sensitive to temperature (T) and pressure (P), which strongly impact membrane fluidity, permeability and membrane-associated functions. To maintain membrane functionalityin response to T or P fluctuations, bacteria alter their lipid composition, a mechanism termed homeoviscous adaptation. Structural differences between archaeal and bacterial membrane lipids raise the question whether hyperthermophilic and piezophilic archaea also regulate their membrane functionality by a similar mechanism.
Methods. The membrane lipid composition of the piezophilic archaeon *Thermococcus barophilus* was determined in sub- and supra-optimal P and T conditions following extraction and separation into apolar and polar fractions. Apolar lipids were analysed by GC and GC-MS whereas polar lipids were hydrolysed before analysis by HPLC/APCI-MS.
Results. In contrast to previous reports, archaeol and caldarchaeol are the major core lipids of *T. barophilus*. The apolar lipids of the strain consist in series of C35 and C40 unsaturated isoprenoid hydrocarbons with a lycopane-like carbon skeleton. Variations in P or T relative to optimal conditions of growth affect both the relative proportions of core lipids and the degree of unsaturation on hydrocarbons, which demonstrate homeoviscous adaptation and a structural role for lycopenes in *T. barophilus*. To accomodate for this lipid composition, we propose a novel membrane model for *T. barophilus* in which lycopenes are incorporated at the interface between the two layers in a mixed bilayer-, mono-type membrane, and are involved in membrane permeability and rigidity regulation [1]. To our knowledge, this is the first report of homeoviscous adaptation in a piezophilic, hyperthermophilic archaeon involving the modulation of the unsaturation level of non-core lipids of the membrane.

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Genomic and physiological analysis of deep-sea luminous, piezophilic *Photobacterium phosphoreum* ANT2200

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Photobacterium are gram-negative bacteria and represent one of the major genera of the family *Vibrionaceae*. They are facultatively aerobic and chemoorganotrophs, and widespread in coastal, open-ocean and deep-sea environments. Recently, we have sequenced the first genome of the species *Photobacterium phosphoreum* and compared it with other nine genomes from six species of the genus *Photobacterium*. The genome of the piezophilic *Photobacterium phosphoreum* strain ANT2200 exhibits a metabolic versatility and is capable of growing in minimal media by deriving energy from fermentation of glucose or maltose, or anaerobic respiration with formate as electron donor and trimethlyamine N-oxid (TMAO), nitrate or fumarate as electron acceptors. Its genome encodes the lux-rib genes, responsible for bioluminescence. We found that the intensity of bioluminescence was proportional to the growth rate, under catabolite repression regulation, but independently on the cell density. As genomic indications for adaption to deep-sea habitats, P. phosphoreum ANT-2200 possesses the hallmark genes that are up-regulated at high hydrostatic pressure in piezophilic *Photobacterium profundum* SS9. Similar as *P. profundum* SS9 the genome of *P. phosphoreum* ANT-2200 encodes four TMAO reductases. High hydrostatic pressure and TMAO induce the expression of these genes differently. These genomic and physiological analyses provide useful clue in the studies of evolution of luminous bacteria and adaptation of bacteria to the deep-sea habitats.

Protein-protein interactions network of genomic maintenance in Pyrococcus abyssi

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The composition and dynamics of the large molecular complexes acting in DNA repair, replication and transcription in bacterial and eukaryotic systems are considerably well described, paving the way towards elucidating the spatial and temporal organization of the DNA metabolisms processes in vivo within the context of chromatin and inside the intact cell. In contrast this vision is fragmentary in Archaea owing to the still moderate efficiency of genetics and in vitro biochemistry combination. To gain insights into genomic maintenance processes in hyperthermophilic archaea, a protein-interaction network centred on informational processes of Pyrococcus abyssi was generated by affinity purification coupled with mass spectrometry. The complexes identified give insights into the connections of DNA replication with recombination and repair, leading to the discovery of new archaeal components and of unsuspected associations between eucaryotic homologs. It provided clues towards the function of new molecular complexes with the potential to better understand genomic maintenance processes in hyperthermophilic archaea. We will also describe our ongoing work aiming to characterize the enzymology underlying the activities of some of the complexes detected, PCNA-MRE11/Rad50 and RPA-RNAP. This interaction map provides a valuable tool to explore new aspects of genome integrity in Archaea and also potentially in Eucaryotes.

High-pressure and functional studies of metabolic enzymes from different deep-sea microbes

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The high-pressure conditions in which microbial life thrive in the deep-sea impact many cellular functions. The discovery of obligate piezo-hyperthermophilic archaeon such as *Pyrococcus yayanosii* suggests the existence of specific metabolic or structural adaptations with respect to high pressure. We examined the effects of high-pressure conditions on the activity, stability, substrate specificity and co factor requirement of enzymes from thermophilic microorganisms isolated at different depth. High pressure imposes diverse constraints on the proteins depending on their 3D structures, oligomeric state and biochemical activities. Three enzymatic families were studied: the malate dehydrogenases from different thermophilic bacteria and the hydroxypyruvate reductases (HPR) and M42 aminopeptidases from thermococcales. We used an integrated approach combining enzymology, biophysics (SAXS) and X-ray crystallography to compare the enzymes properties under atmospheric and high-pressure conditions.
This work revealed that the deep-sea MDH are activated by high pressure. A deeper study on temperature and pressure effects suggested a prevalence of stabilization mechanisms to explain high pressure adaptation in this enzymatic family. On the contrary, the HPR enzymes are stable over a wide range of pressure but high pressure greatly influences the cofactors and substrate specificities of the enzymes. In particular, we showed that NADPH tends to protect the enzymes from high-pressure inhibition. Therefore the cofactor availability may have an importance for the enzymatic activity under high pressure. The M42 TET peptidases were chosen to explore the effect of high pressure on large molecular assemblies. The complex is made of 12 subunits forming a 500-kDa edifice. Surprisingly, the surface and deep-sea enzymes remains very actives even above the highest pressure at which oligomers have been found to collapse.
These studies indicated that the effect of pressure on the proteomes is likely to be very contrasted and that high-pressure adaptation seems to involve enzymatic properties or stabilizing effect depending on the type of enzyme. The evolutionary processes underlying pressure adaptation at the molecular level should therefore be very difficult to detect at the genome scale.

Pressure adaptation of the deep-sea enzymes and discovery of the high-pressure X-ray systems.

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Enzymes from the deep-sea piezophilic microorganisms are adapted to the deep-sea pressure conditions; therefore they are keeping high level of enzyme activities at high-pressure conditions (1). Actually enzymes from atmospheric pressure adapted microorganisms could be inactive under higher-pressure conditions. In the case of respiratory proteins, cell divisional protein FtsZ, RNA polymerase subunit proteins, dihydrofolate reductase (DHFR), and isopropyl malate dehydrogenase (IPMDH), proteins from piezophilic microbes were unique for adaptation to high-pressure environment and some of them were much more stable and active under elevated pressure conditions (2). To elucidate the pressure-adaptation mechanisms of deep-sea microbe's proteins, we have investigated structural studies on these proteins by a high-pressure protein crystallography method using a diamond-anvil cell (3). Using this high-pressure crystallography technique, the details of the enzyme structural features for pressure adaptation could be observed.

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Posters

Development of a viability assay by ATPmetry using a preconcentration filter

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Abstract
For many years, scientific community seeks to apprehend and define limits of Life. In 1977, the U.S. diving submarine Alvin allowed to discover a lush life in wrinkle depth near the Galapagos. Deep-sea hydrothermal vents are characterized by extreme parameters such as temperature, pressure or salt, sulfur and metal concentrations. Studying microorganisms's viability in these marine ecosystems is of main interest for our understanding of life at extremes. Toward the aim of developing of method for studying cell viability in extreme marine habitats, we first developed a protocol for common marine samples, rich in inhibitors.
Cell viability can be detected using a lot of techniques including determination of adenosine-5'-triphosphate (ATP) concentration (Pridmore et al., 1984). This technique has the advantage of being simple, rapid and sensitive (Maehara et al., 1987; Shama,2013; Eydal et Pedersen, 2007). However, these methods of measurement of cell viability are effective on natural media with little or no salt (Hammes et al., 2010).
This work aims to develop a strategy to optimize cell viability measurements in seawater which contains many inhibitors of luciferase, the enzyme used for ATPmetry. We thus focus on the determination of intracellular ATP (ATP-I) using a filtration system to preconcentrate cells. This approach should increase the sensitivity of the ATPmetry method (Veza et al., 2008; Simon et al., 2013).
In this work, considering previous reports about cell filtration (Wang et al., 2008), five filters of various compositions, with different pore-size, were compared in terms of retention capacities, and repeatability of ATP-I measurements.
This has permitted, in particular, to observe the effect of filter composition on measurement of ATP-I, to show the effect of volume ([ATP] = f(filtered volume)), as well as demonstrate the effect of flow. We showed that microorganisms with a low cell volume are better retained by 0.1 µm pore-size filters and therefore assayed. Our results indicated that a significant increase in sensitivity was obtained, but that the 0.1 µm filtration did not allow the retention of all cells.
The second part of this study, still in progress, will be to compare various seawater samples with different concentrations of microorganisms from Oceanopolis's aquarium (Brest, France), to determine if cell concentration (measured by flow cytometry) and ATP can be correlated.
Keyword: ATPmetry, bioluminescence, filtration, seawater, flow cytometry.

Genetic modifications of the hyperthermophilic piezophilic archaeon *Thermococcus barophilus*: Development of a stringent 6-methylpurine counterselection system.

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Studying extremophiles archaea is of major interest for characterizing life adaptation to extremes environments. Despite the new insight about the contribution of the Archaea to the environment, poorly is known about their physiology and diversity in the light of their ecological niches (in term of pH, salinity, pressure and temperature). In order to better understand their ecological role in addition to the great biotechnological potential that archaea represent, the development of genetics manipulations is required in complement to cultural and "omics" approaches. Today, a dozen of the most studied Archaea has been genetically manipulated. Among those, the hyperthermophilic piezophile archaeon *T. barophilus*, model of our laboratory whose genome is annotated and for which clusters of gene regulated by high hydrostatic pressure have been identified through transcriptomic approach.

Based on homologous recombination, a gene disruption system was achieved using simvastatin and 5-fluoroorotic acid (5-FOA) for selection and counterselection respectively. Disruption plasmids were constructed carrying the flanking sequences of each targeted gene and the two selected markers: HMG-CoA and *pyrF* genes that are respectively used for the positive selection (the first crossing over event leading to plasmid integration) and counterselection (the second crossing over event leading to plasmid disruption). However, the 5-FOA recurrently brings a high number of false positives mutants (60-90%) harbouring phenotypes sim^r and 5-FOA^r while true positives are sim^s and 5-FOA^r.

In order to reduce the rate of false positive, a new counterselection system based on toxic adenine analog named the 6-methylpurine (6MP) was done. Thus, *pyrF* has been replaced by TK0664, a *T. kodakarensis* gene responsible for its natural sensitivity to 6MP. Although a homologous gene TERMP_00517 exists, *T. barophilus* has been described as not sensitive to 6MP.

During this study, sensitivity to 6MP was brought to *T. barophilus* through plasmidic disruption system bearing TK0664. Flanking sequences were used to create TERMP_00517 mutant for avoiding possible interfering effect. Liquid growth assays on the different strain constructions at different 6MP concentrations have confirmed the sensitivity of *T. barophilus* to 6MP while refining the proper efficient concentration. In the context of gene disruption on solid media, acting for counterselection, 6MP considerably reduce the rate of false positives (0-30%). Thus, this molecule improves the genetic system for *T. barophilus* allowing efficient future investigations about its cellular process involved in adaptation to extreme environments.