
CIESM Workshop Monographs



Molecular adaptations of marine microbial life to extreme environments

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EXECUTIVE SUMMARY¹ OF CIESM WORKSHOP N°44

1. INTRODUCTION

It is nearly forty years ago (MacElroy, 1974) that the term 'extremophile' was first coined. In the mean time studies of these organisms have progressed fast, to the extent that there are now regular international symposia, as well as scientific journals (*i.e. Extremophiles* and *Archaea* journals), fully devoted to the subject.

Most studies on marine extremophilic microorganisms have been driven by their great potential for the biotechnology industry. These focus on the adaptive mechanisms, at the molecular level, which enable these species to maintain viability in extreme conditions. Another major line of research seeks to define the range of physiological parameters for survival under such conditions, as these may have implications for life elsewhere. It tackles the origin, evolution, distribution of extremophilic microorganisms, and the potential of life to adapt to different environments. Yet, despite the vast published record tracking life in marine extreme environments, very little efforts have been dedicated to the study of these environments from an ecological point of view.

In opening the meeting Prof. Frédéric Briand, Director General of the Mediterranean Science Commission, signaled the fact that the subject of the workshop reflected the increasing importance of molecular ecology and of marine microbiology in the work of CIESM. In recent years the Commission had produced a number of reports on marine biotechnology and on the potential of active molecules. This meeting, gathering invited experts from eight countries, would face the challenge of drawing priorities for research on adaptations of marine extremophiles, with special attention to the rare opportunities provided by the Mediterranean and its unique extreme habitat in this regard. He encouraged participants to make their point on new ways to contextualize marine extremophilic microorganisms.

Frederic Briand warmly welcomed the international participants, inviting them to introduce themselves in turn, and he expressed his gratitude to Dr. Anastasia Miliou, scientific manager of the Archipelagos Institute of Marine Conservation (Greece), for her local logistic support. He then invited Prof. Milton da Costa, Chair of the CIESM Committee of Marine Microbiology and Biotechnology, to detail the conceptual genesis and the objectives of this exploratory seminar.

¹ to be cited as: Giuliano L., da Costa M., Bartolucci S., Bonch-Osmolovskaya E., Meltem Conk Dalay M., Liebl W., Pella F.X., Prangishvili D., Rosselló-Móra R., Sass H. and F. Briand. 2012. Executive Summary of CIESM Workshop n° 44, Samos, Greece, 15 p.

Milton DaCosta outlined how the selected complementary expertises could help filling up the gaps, and he introduced the subject by illustrating some major examples of life adaptations to hypersaline environments.

Dr Laura Giuliano followed, urging the participants to seek new angles in the discussions so as to add value to the fast-growing corpus of knowledge on the subject. As an example, she provocatively encouraged her colleagues to think from the perspective of microbes when listing or identifying ‘extreme’ conditions for survival. Why not view a pure culture as an ‘extreme environment’ for natural microbial populations? And what about the difficult life of a pathogen, preparing to aggress its 'armoured' host organism? Or, from a geographic point of view, are we capable of tracking the frontiers for life on our Planet?

This started a helpful discussion on the boundaries of extremophiles, with Henrik Sass drawing attention to studies related to geo-biosphere system boundaries (including the flows of carbon), while Ramon Rossello-Mora focused on the antropocentric point of view and argued that we are still a long way from deciphering life in marine extreme environments. He proposed to reflect on the feasibility of comparative studies of different marine extremophilic biotopes, and much contributed to the ideas around which this Executive Summary has been developed.

2. GENERAL ECOLOGY OF EXTREMOPHILES

Extremophiles are those forms of life that thrive in ‘harsh’ environments thanks to specific molecular adaptations which allow them to counteract the environmental ‘denaturing effects’. Environmental challenges may include physicochemical stress factors such as pH, salinity, temperature, pressure, radiation, but also elevated concentrations of chaotropic or toxic compounds like magnesium ions, hydrogen sulphide or heavy metals. Another environmental challenge for microorganism is nutrient deprivation or the exposure to long-term starvation (see Sass, this volume).

The niche of an extremophile is typically defined by ‘abiotic’ factor(s) -*i.e.* the so-called ‘extreme’ parameter(s)- rather than by the classical inter-species (grazing *vs.* competition) interaction mechanisms.

Counterintuitively, except in few cases, one finds no ‘monospecific’ niches within extreme environments. On the opposite, these usually harbour rather diversified communities².

Among the exceptions figures *D. radiodurans*, which evolved a unique phenotype capable of resisting massive ionizing radiation³. Another ‘unique’ case is the capacity for anaerobic hydrogenogenic growth on formate shown so far only by the deep-sea group of the hyperthermophilic archaeal order *Thermococcales*. The special complexity of genetic determinants’ systems associated to these ‘unique’ adaptive mechanisms may have prevented their effective lateral transfer.

² Salterns, for example, host microbial communities that are considerably rich in taxa, and are characterized by a stratified distribution along the salinity gradients (see Rossello-Mora: this volume).

³ A dose of more than 12,000 gray is necessary to kill 90% of *D. radiodurans*. This dose is 100 times greater than that required to kill most bacteria and 2000 times the dose that a human being can support (see Pella, this volume).

Whatever the ‘extreme parameters’, the evolution of related adaptive mechanisms looks ‘randomly’ distributed within the three domains of cellular life, although it always favours Bacteria and Archaea. One example is the salt-adaptive mechanism involving the selective influx of potassium ions into the cytoplasm, which is peculiar to three distinct lineages (the bacterial Order Halanerobiales, the archaeal Family Halobacteriaceae and the bacterium *Salinibacter ruber*) (Santos & da Costa, 2002). The redundancy of adaptive strategies within extremophiles can be plausibly explained by convergent evolution, ancient characteristics retained in scattered groups or through massive lateral gene transfer.

In certain extreme environments, viruses are likely to play the role of ‘top-down controlling factor’. Hypersaline environments, for example, are characterized by a very high number of virus-like particles (VLPs) (see Rossello-Mora, this volume). Viruses could also be responsible for accelerated mutation rates (*i.e.* evolutionary shifts), provided that the extremophilic communities did not develop adequate ‘immune system mechanisms’ (*i.e.* CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats) (Marraffini & Sontheimer, 2010). Studies on the ‘metagenomic resilience’ of VLPs-rich extreme environments could target, for example, the occurrence of CRISPR-like families in those environments.

3. ‘EXTREME’ VS. ‘ANCIENT’ CHARACTERS

Before there was life (even in its simplest imaginable embodiment), there must have been “pre-biotic” chemistry that has to be analysed from chemical, geo-chemical, and geophysical perspectives. The best window into the earliest stages of life evolution might be provided by the conservation of the fundamental intracellular chemistry. All cells contain much more potassium, phosphate and transition metals than modern or reconstructed primeval oceans, lakes or rivers. Assuming that the first cells did lack sophisticated membrane pumps, this ion composition is expected to reflect the ion composition of the habitats of protocells. The chemistry of basins at anoxic geothermal fields would resemble the internal milieu of modern cells. Geochemical reconstruction shows that the ionic composition conducive to the origin of cells could not have existed in marine settings but is compatible with emissions of vapour-dominated zones of inland geothermal systems. In contrast to the fluids of deep-sea vents, the geothermal vapour is enriched in phosphorous and boron compounds that would be essential for the emergence of the first RNA-like oligomers. The precellular stages of evolution might have transpired in shallow ponds of condensed and cooled geothermal vapour that were lined with porous silicate minerals mixed with metal sulphides and enriched in K(+), Zn(2+), and phosphorous compounds. The hypothesis described here implies that cells invaded the oceans at a relatively late stage of evolution, after elaborate, modern-type membranes capable of efficiently maintaining ion gradients had evolved. Thus, life might have originated in an isolated location and became a planetary phenomenon only after the colonization of the oceans (Mulkidjanian *et al.*, 2012).

Metabolic pathways may have been assembled by the recruitment of primitive enzymes that could react with a wide range of chemical related substrates. Such relatively slow, unspecific enzymes may have represented a mechanism by which primitive cells with small genomes could overcome their limited coding abilities. Accordingly, new enzymes with narrow specificities would result from gene duplication and divergence events (Copley, 2003).

Recent computation comparisons of full genomes show that the translation system is the only

complex ensemble of genes that is conserved in all-extant cellular life forms. A few genes of this system⁴ have become the standard for life detection on Earth, including microbial life in a wide range of terrestrial environments, such as the extreme environments (Isenbarger *et al.*, 2008). Given the extraordinary conservation of the translation system, comparison of orthologous sequences reveals that the core of the translation system was already fully shaped in Last Universal Common Ancestor (LUCA) (Anantharaman *et al.*, 2002).

Despite rather common belief, there is no proof that extremophiles are living ‘relicts’ of the earliest forms of cellular life on Earth. On the other hand, marine extreme environments can exhibit accelerated evolutionary patterns due to the expression of efficient natural competence apparatus -typical of some extremophilic microorganisms (*i.e.* *Thermococcus* spp.)- and fast viral infective cycles. According to the mapping of some adaptive ‘extremophilic’ features upon the genealogical ancestry, molecular adaptations to extreme conditions seem to have arisen and accumulated independently multiple times in separate lineages. The oldest yet persisting extremophilic character known to date is the molecular basis of thermophily as it is expressed in some hyperthermophilic Bacteria (*i.e.* *Aquifex*, *Thermothoga*).

Horizontal gene transfer has also facilitated, at times, the acquisition of extremophilic characters in eukaryotes. For example, the unicellular red alga *Galdieria sulphuraria* has acquired horizontally at least 75 bacterial and archaeal protein-coding genes, which are involved in processes ranging from heavy-metal detoxification to glycerol uptake and metabolism (Schonknecht *et al.*, 2013).

4. ADAPTING TO ONE, TWO OR MORE ‘EXTREME’ PARAMETERS

Scientific reviews of the ingenious adaptations for survival adopted by these fascinating organisms provide plausible hypotheses to explain the simultaneous occurrence of different adaptive mechanisms in a single organism. Briefly, ‘stress protectant’ mechanisms may co-exist if they are structurally ‘compatible’, and this happens only when simultaneous stresses cause similar effects on the exposed organism (*i.e.* high pressure / cold adapted ‘psychropiezophiles’; halo-thermophiles; radiation / desiccation / oxidation tolerant microorganisms) (Daly *et al.*, 2004; 2010). The contradictory effects of high temperature, high salt and acidic stresses on the functional structure of the protonic pump have probably prevented ‘halo-acido-thermotolerant’ mechanisms to co-exist. However, the existence of halophilic alkalithermophiles has been proven (Poosarla *et al.*, 2010; Mesbah & Wiegel, 2012). In line with this principle, the most challenging ‘polyextreme’ environments are those that may harbour adaptive mechanisms that have not been described to date (*i.e.* Dallol hot springs, Ethiopia) (Darrah *et al.*, 2013).

5. GEOMICROBIOLOGY : MAPPING THE LIMITS OF LIFE ON OUR PLANET EARTH

There is hardly a niche on Earth that has not been colonised. Life can be found in scalding, acidic hot pools, in the driest deserts, and in the dark, crushing depths of the ocean. It has been even found in Polar regions and in toxic dumps. Virtually no subsurface environments can be assumed to be sterile *a priori*. Instead, subsurface environments may host

⁴ The ribosomal genes (16S and 23S) and few other universally conserved nucleotide sequences in genes encoding particular classes of transfer RNAs and within the nucleotide binding domains of ABC transporters are the most conserved DNA segments across the kingdoms of life.

microorganisms, which may be viable but inactive, even over geological time scales.

The list of the ‘most extreme’ forms of life is regularly revised along with new scientific outputs. To date, although the record-setters are subject to debate, the Earth's toughest organisms include the top ‘salt-loving’ microorganism ‘*Haloarcula marismortui*’ (Oren *et al.*, 1990), an archaeon (family Halobacteriaceae) living in the Dead Sea, the saltiest body of water on Earth (eight times saltier than the ocean). Haloarchaea become entrapped and survive for long periods inside halite (NaCl) crystals. They are also found in ancient subterranean halite (NaCl) deposits, leading to speculation about survival over geological time periods (Grant, 2004). The hottest growth limits belong to the obligate litho-autotroph, iron-reducing archaeon *Geogemma barossii* (Kashefi & Lovley, 2003), growing at temperatures from 85-121°C and originally living in a deep-sea hydrothermal vent in the Northeast Pacific Ocean. Due to its specialised cell membrane that does not stiffen in frigid temperatures⁵, and to the production of antifreeze (cold-shock domain) proteins, the gamma proteobacteria *Colwellia psychrerythraei* (Deming *et al.*, 1988) strain 34H can withstand -196°C, the temperature of liquid nitrogen (without any cryoprotecting agents added). This bacterium is able to grow under deep-sea pressure and maintain swimming capabilities at temperatures of -10°C in its natural habitat of the deep sea Arctic and Antarctic Ice, the coldest marine habitat on Earth. Intense solar radiation (due to the UV component of solar light) has usually very dramatic effects; *Deinococcus radiodurans* is among a small number of bacterial species that are extremely resistant to ionizing radiation, UV light, toxic chemicals, and desiccation (Kriško & Radman, 2010). The rocks beneath the sea appear to be teeming with life. The deepest living cells- many of which are Archaea from the genera *Pyrococcus* and *Thermococcus* have been found under the seabed off Newfoundland (Canada) in sediments 111 million years old, 1.6 kilometres beneath the sea floor (Russel *et al.*, 2008). Acidic hot springs, geysers and the acid runoff from mines harbour acidophilic microorganisms, of which the most ‘extreme’ representative is *Picrophilus* (with some species growing down to a pH of 0). The most alkaline environments in the world are soda lakes, which can have a pH as high as 12, akin to ammonia. A number of microbes enjoy these caustic conditions, including *Natronomonas pharaonis* which was first isolated from soda lakes in Egypt and Kenya in the 1980s (Soliman & Trüper, 1982). Microbes can thrive in hazardous waste dumps, mine tailings, toxic sludge of heavy metals like zinc, arsenic and cadmium. *Geobacter* bacteria (Lovley *et al.*, 1993), for example, convert dissolved uranium into a solid form.

According to the published record, the availability of water in the surrounding habitat is one of the most important parameters affecting the survival and growth of organisms, which shall maintain their cell turgor pressure within well-defined limits. Water is thought to be crucial for life because it provides a medium for nutrients to diffuse into cells and wastes to drift out, and a solvent for critical metabolic reactions.

Hypersaline sites (*i.e.* salterns, deep hypersaline basins), where water availability is limited by a high concentration of salts (usually NaCl) are essentially microbial habitats, almost exclusively populated by prokaryotes, either using the ‘salt-in’^{6,7} or the ‘compatible solutes’ (Oren, 2008) strategy to counteract the osmotic stress (see Box I).

⁵ Psychrophilic membranes usually contain polyunsaturated fatty acids and cis-isomerases, which aid in the cell membrane fluidity.

⁶ Anaerobic halophilic alkalithermophiles are probably using an additional strategy or strategies to adapt to high salinity (see Mesbah & Wiegel, 2012).

⁷ Halophilic enzymes are active at high salt concentrations. They are characterized by a high content of acidic amino acids (*i.e.* glutamate, aspartate) that infer an excess of negative charges to the protein surface.

Box I : Osmo-adaptation

Prokaryotic osmoadaptation can be achieved via two different strategies: (i) The 'compatible solute strategy' is to exclude salt from the cytoplasm and to synthesize and/or accumulate organic 'compatible' solutes that do not interfere with enzymatic activity in the cytoplasm (*i.e.* glycine betaine, ectoine and other amino acid derivatives, sugars and sugar alcohols). Few adaptations of the cells' proteome are needed, and organisms using the 'organic-solutes-in strategy' often adapt to a surprisingly broad salt concentration range. Most halophilic Bacteria, but also some halophilic methanogenic Archaea use such organic solutes. (ii) The "salt-in" strategy, involves accumulation of molar concentrations of potassium and chloride, so that the cytoplasm becomes isosmotic with the extracellular medium. In contrast to Na^+ , a high concentration of intracellular K^+ is not toxic to these cells. This method, which is commonly used by the most extreme halophiles, relies on specially featured cationic pumps that eject Na^+ (*i.e.* based on Na^+ -cotransport mechanisms or electrogenic Na^+/H^+ antiporter associated to the massive K^+ intracellular accumulation). It goes on with the synthesis of 'halophilic enzymes'.

Whereas the functional biosphere may primarily be constrained by water activity, the mechanism(s) by which this occurs have not been fully elucidated. The water dynamics inside microbial cells adapted to thrive in low water activity environments was studied by means of neutron scattering using spectrometers. Results showed that the presence of NaCl (3.5M) inside the 'hyper-halophilic' cells significantly reduced the speed of the cell water. This unique water movement relies on a water structure that seems to facilitate the binding of K^+ with the cells exhibiting a slower water movement (Tehei, *et al.*, 2007).

According to certain scientific hypotheses, water activity is not the definitive parameter that dictates the limits of microbial activity in all environmental niches. For various xerophilic organisms, water activity per se does not limit cellular function while other solutes activities- most notably chaotropicity that weakens macromolecular interactions and disorders cellular structures- can determine the biotic window. Sometimes the addition of some compounds to the system may reduce the net chaotropicity (Hallsworth *et al.*, 2007; Williams & Hallsworth, 2009). Some fungi grew optimally under chaotropic conditions, providing evidence for a previously uncharacterized class of extremophilic microbes. Further studies to elucidate the way in which solute activities interact to determine the limits of life may lead to enhanced biotechnological processes, and increased productivity of agricultural and natural ecosystems in arid and semiarid regions.

6. BETWEEN LIFE AND DEATH – VIRUSES OF EXTREME ENVIRONMENTS

Since they usually depend on a cellular host for replication, viruses have been considered as not really alive (*i.e.* "fragments of life") for a long time. Despite the general difficulties in studying them, our knowledge of viruses in extreme environments has considerably increased over the past few years. Like all other organisms, extremophiles serve as hosts for viruses, the smallest infectious biological entities known to date. A recent study showed that not all viruses are functionally inactive once they are outside their host cell. Particularly, a virus that infects a hyperthermophilic archaeon growing in acidic hot springs develops a very long tail at each of its pointed ends after being released from its host cell. The process, which occurs only at the temperature of the host's habitat (75–90 °C) and does not require an exogenous energy source or any cofactors, may be a strategy for viral survival in an environment that is unusually harsh and has limited host availability (Håring *et al.*, 2005).

Viruses can strongly influence biochemical adaptations required for life in extreme marine environments. For example, prophage-like elements in microbial genomes may sometimes act as gene transfer agents (GTAs) and so enhance horizontal gene transfer in marine extreme

environments. Prophages may also directly contribute to the survival of their host in unfavorable environments by suppression of unneeded metabolic activities (Paul, 2008).

Molecular-based surveys showed that in solar salterns the viral population abundance and diversity increased along the salinity gradient from near seawater (4%) to saturated sodium chloride brine (37%) (Sandaa *et al.*, 2003). Virus-to-bacteria ratios in Arctic sea ice were among the highest reported in natural samples (Maranger *et al.* 1994). Nevertheless, since viruses isolated from extreme marine environments exhibit exceptional life cycles, estimates based on the classical detection and isolation approaches (*i.e.* those built on the virus' ability to lyse host cell) are most likely biased (see Pranshgivili, this volume).

All viruses isolated so far from extreme environments are double-stranded DNA viruses with moderate genomic complexity (the genome size range from 14 to 80 kb). It is conceivable that this very stable form of genome may be necessary to face the harsh constraints of extreme habitats. It could also explain why no RNA virus has been isolated yet, especially from hot environments. The study of biology and ecology of new viruses isolated from extremophile environments may shed light on the early biological processes as well as on viral evolution (Le Romancer *et al.*, 2007).

7. EXTREMELY HARD TO BE STUDIED – EXPERIMENTAL INSIGHTS

While chemical (including molecular) analytical technologies have progressed at an amazing speed, environmental sampling still represents a technological 'bottleneck'.

This is especially true with regard to the exploration of harsh environments, which are often populated by highly specialized microbial populations that require special care for being preserved and maintained.

Moreover, the unusual ecological patterns that shape most extreme environments have evolved undisturbed through very large (*i.e.* geological) temporal scales. Hence, not only the resident microbial populations but also the hydrology and geochemistry of most extreme environments are likely to be particularly sensitive to changes or perturbations in the system (low resilience capacity).

Investigations on marine extremophilic microorganisms should therefore incorporate techniques that eliminate, to the degree possible, any contamination of the source environment due to sampling/drilling. In case of viable microbial samples (*i.e.* with the aim to proceed further with incubation/cultivation of sampled microbes), sampling devices should allow to maintain both the indigenous microbiology and geochemistry (*i.e.* geochemistry and transport properties of the host environment; hydro-geological properties, etc.).

Technical limitations in isolating and culturing marine extremophilic microorganisms affect significantly studies related to the viruses that inhabit marine extreme environments. Those, in fact, rely mainly on the use of host-cell cultures. While databases and molecular-based resources for studying function in uncultured bacterial communities are relatively advanced, far fewer exist for their viral counterparts. The current repertoire of viral gene sequences is still very limited, which is remarkable considering the extreme abundance and diversity of viruses revealed over the past decade by metagenomic analysis. Moreover, temporal genetic changes of viruses may often be greater than spatial changes or change between viral species.

The distinction of ‘pro-active- viruses against ‘remains’ or ‘ghosts’ is another complicated issue that requires substantial technical improvements. Verifying the viral nature of virus-like particles and identifying their hosts will require further study. As a consequence, nothing is known, yet, of the virus-host dynamics that characterize marine extreme environments. New tools enabling the construction of marine viral metagenomic datasets appropriate for quantitative viral ecology are necessary.

More generally, innovative datasets will require standardized data formats located in specialized databases, referenced metadata (location -including geochemical settings, methodology, sequencing technology, assembling and annotation platforms, etc.) so as to enable downstream analyses.

As potential terrestrial analogs of other planets, marine extreme environments could benefit of the most refined technologies yet in use to carry out research in astrophysics and astrobiology⁸ and, in return, provide those sectors with optimal test case conditions.

8. MARINE BIOTECHNOLOGIES – THE POTENTIAL OF THE MEDITERRANEAN REGION

Without doubt, the major impetus driving extremophile research is their associated biotechnological potential. Indeed, the application of extremophiles in industrial processes has opened a new era in biotechnology. Each group has unique features that can be exploited to provide biomolecules with a wide variety of applications

From a commercial perspective, enzymes from extremophiles – extremozymes – have made the most impact so far. As an example, alkaline proteases, derived from alkaliphilic species, constitute an important group of enzymes that find applications primarily as protein-degrading additives in detergents. Thermo-alkali-stable, cellulase-free xylanase have important applications in biobleaching (Kumar & Satyanarayana, 2012). Given the robust nature of alkaliphiles, these enzymes can be subjected to harsh treatments, including elevated temperature, high pH, surfactants, bleach chemicals, and chelating agents, where applications of many other enzymes are limited because of their low activity or stability. The fact that enzymes and protein complexes from extremophiles are easier to crystallize make them good models for structural biology.

The Mediterranean region presents a number of challenging specificities, which make it a ‘hotspot’ for bioprospecting. Due to very peculiar geological settings, and a complex history characterized by a long series of catastrophic events, the Mediterranean Sea (2.5 million square km body of water; average depth of 1,500 m ; maximum depth of 5,267 m)⁹, features high species richness, exceptional concentrations of endemic species and a diversity of easy-to-reach extreme environments that harbour unique forms of life (particularly microbes) and remarkable ecological processes. High marine biodiversity means a very distinct set of gene pools and natural products with large potential application in medicine, food development and bio-energy (See Box II).

⁸ Astrobiology is the study of the origins, evolution, distribution, and future of life in the Universe.

⁹ Calypso Deep (Ionian Sea)

Box II – Taxonomic provenance of Marine Genetic Resources (MGRs)

While the taxonomic origin of MGRs covers the entire spectrum of the "Tree of Life", marine invertebrates are increasingly selected for the screening of bioactive marine natural products (MNPs) to develop new therapeutic agents (Hill & Fenical, 2010). Among them, sponges, the major contributing species, have emerged some 700–800 million years ago. This early appearance allowed them to develop diversified, complex, and advanced defence systems against their pathogens and predators through specific MNPs (Belarbi *et al.*, 2003; Sipkema *et al.*, 2005). While the number of active compounds obtained from marine microorganisms (including actinomycetes, fungi, prokaryotes and phytoplankton) has only doubled from roughly 50 in the 1980s to over 100 in 2008 (Hu *et al.*, 2011), their huge reservoir of new biosynthetic genes (with emphasis on microbes inhabiting 'extreme' marine environments such as hydrothermal vents and hypersaline lakes) found its way into patents at a much faster rate. So far marine natural products have been mostly derived from large sessile organisms which are easily collected and provide relatively large amounts of biomass for screening of natural products (Arrieta *et al.*, 2010). In fact, more and more potential novel compounds isolated from marine invertebrates turn out to be biosynthesized by mutualistic microorganisms (Sabdono & Radjasa, 2008). Advances in molecular biology (including high throughput sequencing, metagenomics, and bioinformatics), plus easy industrial culturing and the application of gene technology, are making marine microbiology the most promising field for drugs development.

As the Mediterranean Sea borders three continents, it interacts with highly diversified economic and socio-cultural systems that range from medium to high human development standards (HDR UNDP, 2013)¹⁰. It therefore offers a challenge and great opportunity to design new models of collaboration and marine co-governance with a view to better secure the various user communities' needs.

9. THE MEDITERRANEAN 'EXTREMOPHILES' BIOGEOGRAPHY -

Mapping biodiscovery hotspots of Mediterranean marine natural products

Biogeography has been defined as the "science that attempts to document and understand spatial patterns of biodiversity" (Brown & Lomolino, 1998). The biogeography of extremophiles is receiving increasing attention because they are one of the richest sources of novel bioactive compounds with industrial applications.

Whether or not marine microorganisms are capable of exhibiting biogeographic patterns is a topic of heated debate in the literature. On the opposite, there is no doubt on the geographic traceability of marine *extremophilic* microorganisms since their distribution matches the location of 'extreme environments'.

Mediterranean habitats on the sea floor include sediments of varying geology, seamounts, banks, canyons, slopes, trenches, ridges, mud volcanoes, gas seeps, carbonate mounds, saturated brines and hydrothermal vents. Those habitats harbour unique biological communities, most of which are 'extremophilic'.

Detailed geographic location of extreme environments (*i.e.* the most promising areas for bioprospecting) in the Mediterranean shall facilitate natural product (MPNs) search and discovery strategies. By improving accessibility to marine genetic resources of great potential for industry, while ensuring a fair distribution of benefits to the source countries populations,

¹⁰ The Human Development Index (HDI) integrates health, education and living standards into a single statistics, providing the United Nation Development Program (UNDP) with a common frame of reference for both social and economic development. The HDI sets a minimum and a maximum for each dimension, called goalposts, and then shows where each country stands in relation to these goalposts, expressed as a value between 0 and 1. Regular UNDP Reports (from 1993 to date) provides specific goals for poverty reduction and human development, plans and approaches for national, regional and international action. <http://hdr.undp.org/en/statistics/hdi/>

this effort shall contribute to the development of marine biotechnology in the region. It may also help adequate planning for integrated maritime management as to space competition within various users (tourism, energy, shipping etc.).

Maps shall address the taxonomic details of MPNs source organisms, the chemical types of isolated compounds, the location and physical / chemical (including geo-chemical) properties of sampling sites.

10. CONCLUDING REMARKS

Studies on life in marine extreme environments have been largely driven by the high potential of extremophilic microorganisms for various industrial purposes. Not surprisingly, most investigations focus on the adaptive mechanisms typical of microbes thriving in extreme environments. Far fewer studies have been devoted, till now, to understand the ecology of extreme environments, and when they do, the work tends to be fragmented, category by category (*i.e.* hydrothermal vents, hypersaline lagoons, extreme acidic environments etc.).

To our knowledge, this Monograph represents one of the first attempts to analyse the ecology of marine extreme environments globally, by identifying common trends and features. This approach easily illustrates the potential of marine extreme environments to meet fundamental, challenging questions on the evolution, or the limits of life on our planet and beyond. It also points to fundamental gaps of knowledge regarding inter alia:

- the evidence on Earth of new polyextremophiles which would enlarge the boundaries of Life (*i.e.* ‘halo-acido-thermophiles’?);
- the evolutionary patterns in extreme conditions, and the role of viruses;
- the phylogenetic relationships of analogous marine extreme environments, given that a few pioneering studies have been published on marine deep sea hypersaline basins (for ex. : Antunes *et al.*, 2011 ; CIESM, 2003 ; Edcomb *et al.*, 2009 ; Van der Wielen *et al.*, 2005).

Such investigations will require important technological progress, with special emphasis on the capacity to collect and maintain environmental samples. Because of the limited experimental tools, viruses in marine extreme environments are especially understudied. A major goal would be to set up adequate tools enabling the construction of marine viral metagenomic datasets.

The invited experts concluded that the Mediterranean region is optimally suited for this research area. Further the expected synergies with the marine biotechnology sector hold promises for the economic growth of the region. A concrete suggestion is to start with mapping efforts, identifying the exact location of marine extreme environments in the Mediterranean Basin, with associated useful descriptors, such as physical / geo-chemical properties of sampling sites.

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