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Extremophilic Archaea in Astrobiology

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Abstract. Extremophiles are organisms capable of adapt themselves, survive and thrive in hostile habitats that were previously thought to be adverse for life or lethal. Extreme conditions drive the evolution of their inhabitants, highlighting the role of extremophiles as models for the study of the origin and evolution of life on Earth. Indeed, the investigation of both the microbial communities populating these environments and their biocatalysts, as well as of gene expression control by translational recoding in Archaea provide key insights into the boundaries of life, allowing us to speculate mightily about possible extraterrestrial life form. In this framework, we report here recent advances of our research groups in the astrobiology investigation.

Key words. Extremophiles – Archaea – Hyperthermophiles – Astrobiology

The study of how life originated on our planet is fundamental in astrobiology and is addressed by multidisciplinary approaches. Prebiotic chemistry, aiming to understand the events and the molecular mechanisms that allowed to simple organic molecules to self-assembling in complex polymers containing genetic information, exploit the combination of experimental and computational methods based on the Miller-Urey pioneering experiment reactions (Miller 1953). In principle, the understanding of how molecules combined on Earth does not imply that elsewhere in the universe life shows the same characteristics of the terrestrial one. However, the rich chemistry of carbon suggests that also astral life would be based on this element and that the rules and mechanisms governing life on Earth would be similar elsewhere in the universe. Similarly, the study of extant life is the only way to understand the mechanisms at its basis and to predict the conditions that may have allowed the emergence of life elsewhere in the universe. Therefore, to understand the characteristic of the protocells that firstly colonized our planet and of the Last Universal Common Ancestor (LUCA) of all the living forms on Earth is based on the study of the extant organisms. Despite the great advantage of having Earth as a giant laboratory for the study of the origin of life, finding evidence of how primitive forms of life took place is not easy. Extant organisms are best adapted to the environment after millions of years of evolution

and, even the simplest virus or prokaryotic cell cannot be considered primitive. Therefore, tracing back the ancestors of these modern organisms requires the combination of different tools. Biochemistry and molecular biology offer the possibility to uncover primitive traits in known metabolic pathways, in biocatalytic mechanisms, and in the processes dedicated to store and transfer genetic information; molecular evolution to reconstruct the phylogenetic tree of life; geochemistry and microbiology to define the conditions of early Earth amenable to host life. The identification of the forms of life and of the environments on Earth that may have maintained more primitive traits and can be used as model systems to study the appearance of life is therefore of utmost importance for Astrobiology studies. In these regards, about 40 years ago, the discovery that extreme environments could host life opened a completely new perspective to define the limits of life. Moreover, the discovery that most extremophiles belonged to a new Domain of life previously unknown, the Archaea, distinct from Bacteria and Eukaryota, had a revolutionary impact on modern biology (Woese et al. 1990). Extremophiles distinguish from extremotolerant organisms since the former, to optimally grow, require conditions considered incompatible with human life. In contrast, extremotolerant species can resist to extreme conditions for certain periods of time, often encountering reversible morpho-physiological changes (i. e. sporulation in bacteria or quiescence in invertebrates), then reprising the normal life cycle in permissive conditions. The discovery of extremophiles revealed that the habitats for life are much wider, spanning from -20°C to + 113°C in the stratosphere and deep-sea hydrothermal vents, respectively, in ≤ 120 MPa for hydrostatic pressures in the deep sea, in water activity ≈ 0.6 (more than 5 M salt) in saline lakes and in a range $\approx 0.0 < pH < 11$ in acidic and alkaline biotopes. For astrobiology, the discovery extremophiles had a tremendous impact on the search of extant or past life in the universe and for the study of the origin of life. In fact, the analysis of phylogenetic trees built from the 16S rRNA sequences showed that Bacteria and Archaea growing optimally at temperatures > 60°C populate the deep part of the tree (Figure 1).

This evidence, and geological records indicating a primitive Earth rich of hydrothermal vents, support the hypothesis that extremophiles are the extant forms of life closest to LUCA. However, different hypotheses on the nature of LUCA are still objects of controversies [for a review see Weiss et al. (2018)], which are difficult to test since no fossil nucleic acids are available and experimental approaches are limited to extant (hyper)thermophiles. Notwithstanding these considerations, extremophilic Archaea and the hydrothermal sites hosting them are ideal model systems for astrobiology, allowing to investigate at molecular level possible physiological primitive traits and, in the same time, to correlate their growth with environmental conditions and changes. Often extremophiles find their ideal habitat when a combination of extreme conditions is present. This is the case of the thermoacidophilic archaeon *Saccharolobus solfataricus* (previously *Sulfolobus solfataricus*) optimally growing at 80°C and pH 5.5, which was originally isolated from the Solfatara Pisciarelli (Agnano, Naples Italy) (Zillig et al. 1980) and has been studied in detail in our lab (Cobucci-Ponzano et al. 2011; Cobucci-Ponzano & Moracci 2012; Strazzulli et al. 2017a, 2019). In *S. solfataricus*, also thanks to the funding from the Italian Space Agency (ASI) (*project MoMa, 2006-09, contract n. 1/014/06/0*), we identified a *translational recoding* event known as -1 programmed frameshifting (-1PRF).

In translational recoding, which has been found in all domains of life, programmed deviation of the ribosomes from standard translational rules take place. In *S. solfataricus*, we demonstrated that a gene encoding for the enzyme α-L-fucosidase is expressed by a programmed backward shift of the ribosome on a specific nucleotides sequence allowing to produce a complete polypeptide from two Open Reading Frames (SSO11867 and SSO3060) separated by a -1 frameshift, see Figure 2 (Cobucci-Ponzano et al. 2003b; Rosano et al. 2004; Cobucci-Ponzano et al. 2006a,b, 2008). This was the first report on -1PRF in Archaea

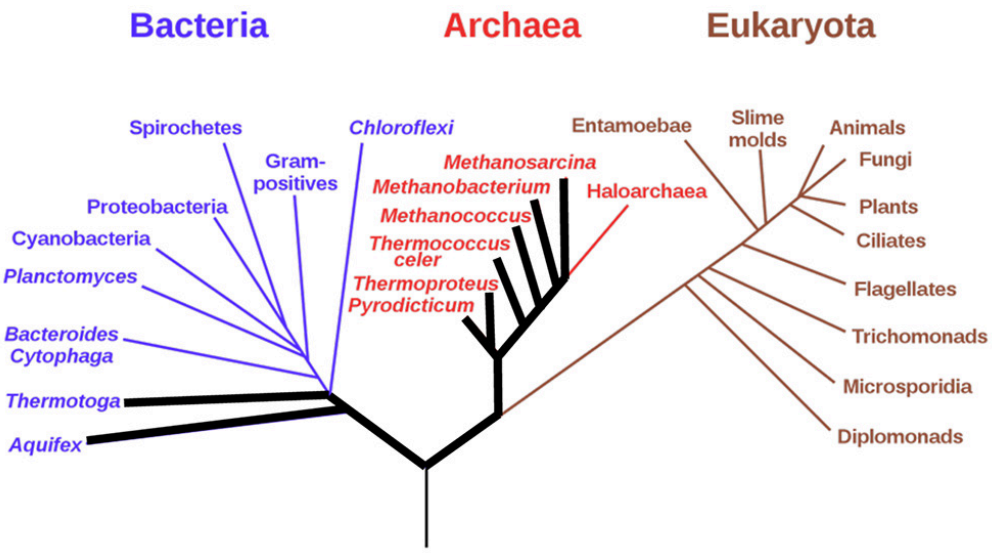

Fig. 1. Phylogenetic tree based on 16S rRNA sequences. The thick lines indicate organisms growing at temperatures $> 60^{\circ}C$.

and demonstrated that this recoding mechanism is universal. Later, we showed that genes putatively expressed by translational recoding could be common in *S. solfataricus* (Cobucci-Ponzano et al. 2010c) and we proposed that this might be a primitive trait maintained through the evolution of the genetic code (Di Giulio et al. 2014).

In Archaea, translational recoding has been demonstrated only for stop codon readthrough, with the insertion of the unusual amino acids selenocysteine and pyrrolysine, and for -1PRF. More recently, it has been suggested that the flexibility of the genetic code and of its decoding may increase microbial fitness under certain conditions (Ling et al. 2015). This could be particularly relevant in extreme environments, which, contrary to common believe, are not immutable but subjected to sudden changes that greatly, and temporarily, modify the chemical-physical parameters to which microorganisms must adapt. For these reasons, the identification and study of interrupted genes in extremophilic Archaea is important from an astrobiological point of view, providing new information on the limits of life on Earth and beyond. To this aim, supported by the ASI project *Life in Space, 2018-21, contract n. 0008788*, we are investigating on the expression by translational recoding of the α-L-fucosidase gene in different growth conditions, to evaluate if its expression can give an advantage (De Lise et al manuscript in preparation). In addition, the analysis of two metagenomic data sets obtained by sampling Solfatara Pisciarelli revealed novel possible recoded genes in the microbiome of this extreme environment. Our data confirm that interrupted genes are widespread in archaeal microbiomes, suggesting that they could be maintained interrupted for evolutionary reasons and that their expression by recoding could be physiologically advantageous under extreme conditions. The majority of hyperthermophilic microorganisms are unculturable and metagenomic of extreme environments is a key approach to exploit these complex microbial populations, providing the access to far more microbial diversity than genomic approaches. In addition, the i*n-silico* screening of (meta)genomic data is a powerful tool to identify, produce in recombinant form and characterize hyperstable enzymes (thermozymes) of biotechnological interest. In fact, thermozymes can re-

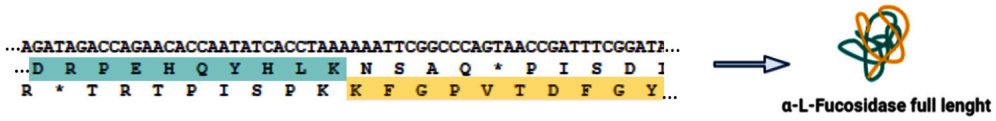

Fig. 2. The *N*-terminal SSO11867 (highlighted in green) in the zero reading-frame and the C-terminal SSO3060 in the -1 reading-frame (highlighted in yellow). Translation by -1PRF led to the production of a full length α-L-fucosidase.

sist to the harsh conditions encountered in several industrial applications (biorefineries, biocatalysis and biosynthesis in organic media and detergents, like cloths/dishwashing, textures treatments, pulp and paper production, etc.) (Cobucci-Ponzano et al. 2003a, 2010d; Iacono et al. 2016). Also thank to the support of previous ASI projects (*MoMa, 2006-09, contract n. 1/014/06/0*, and *Exobiology and extreme environments, 2014-16, contract n. 2014-026-R.0*), an extensive amount of data could be obtained on several thermozymes from extremophiles, mostly Carbohydrate Active enZYmes (CAZymes) by (meta)genomic approaches (Moracci et al. 1994; Prisco et al. 1995; Aguilar et al. 1997; Sunna et al. 1997; Guagliardi et al. 2000; Moracci et al. 2000; Cobucci-Ponzano et al. 2003b; Lauro et al. 2006; Di Lauro et al. 2008; Maurelli et al. 2008; Cobucci-Ponzano et al. 2010a,b; Ferrara et al. 2014; Cobucci-Ponzano et al. 2015; Curci et al. 2019; Iacono et al. 2019; Strazzulli et al. 2020) and their modification by site-directed mutagenesis for structure/function studies and to produce variants with improved ability in the synthesis of oligosaccharides (Guagliardi et al. 1987; Moracci et al. 1994; Prisco et al. 1995; Aguilar et al. 1997; Sunna et al. 1997; Moracci et al. 1998; Guagliardi et al. 2000; Moracci et al. 2000; Trincone et al. 2000; Moracci et al. 2001; Cobucci-Ponzano et al. 2002, 2003b; Perugino et al. 2004; Lauro et al. 2006; Perugino et al. 2006; Di Lauro et al. 2008; Maurelli et al. 2008; Cobucci-Ponzano et al. 2009, 2010a,b,e; Ferrara et al. 2014; Cobucci-Ponzano et al. 2015; Curci et al. 2019; Iacono et al. 2019; Strazzulli et al. 2020).

Recently, we reported the identification and characterization by using a metagenomic enzyme discovery approach from a sample of the Pisciarelli Solfatara (92°C; pH 1.5), of a gene encoding for a novel biocatalyst. This gene has been identified in a contig containing only three hypothetical proteins including a hypothetical sugar phosphate isomerase and an MFS transporter homolog to the sequences from *Sulfolobus sp.* A20 (83%) and *Candidatus Bathyarchaeota archaeon* (47%), respectively (Figure 3), that could belong to a chromosome of a new, unclassified archaeon. This work demonstrates that the enzyme belonging to the family of glycoside hydrolase 109 of the CAZy database classification (Lombard et al. 2013) including α-*N*-acetylgalactosaminidases and β-*N*-acetylhexosaminidase, represents the first archaeal members of this family (Strazzulli et al. 2020).

The cultivation of extremophiles is often hampered by the difficulties of reproducing in the laboratory the harsh conditions of the environments. For this reason, the number of isolated and characterized archaeal strains is generally very low if compared to bacteria. In these regards, the metagenomic approach is often the only alternative to access to a higher number of sequences to study the physiology of extremophilic Archaea. We recently embarked in the sampling of extreme environments to purify metagenomic DNA. Deep sequencing and annotation gave a number of metagenomic libraries from solfataric fields and hydrothermal vents (Menzel et al. 2015; Antranikian et al. 2017; Strazzulli et al. 2017b, 2020).

Comparative metagenomics is also a powerful tool to investigate on the composition of the microbiome populating extreme environments, how living cells affect the site, for instance by producing gasses or exploiting min-

Fig. 3. The genomic environment of the novel archaeal GH109.

erals and nutrients for growth, and how geochemical changes influence the microbiome itself. Indeed, in 2020 we reported on a comparative metagenomic study, supported by the ASI project *Life in Space, 2018-21, contract n. 0008788*, focused on three novel sites formed as a result of recent geothermal activity in Pisciarelli solfatara (Iacono et al. 2020). This area, which is affected by sudden geothermal changes, continuously generate hostile environments for survival and growth of (hyper)thermophilic microbial life forms. This spatial study demonstrates that these sites, although very close to each other, showed different geochemical features that were reflected by significant differences in the dominant microorganisms populating each site and in the number of sequences having no match in the NCBI nucleotide database (Figure 4).

Our study suggested that the selective pressure of these extreme environments might promote the development and conservation of a collection of metabolic and physiological adaptations, which could play a key role in ensuring the presence and persistence of life to those conditions. In addition, we revealed a broad suite of Carbohydrate Active enZYmes correlated to the abundance of lignocellulosic biomasses present around the Pisciarelli area, which represents a considerable carbon source for the microorganisms populating the mud pools. The presence of highly sophisticated mechanisms of adaptation together with the availability of specific biochemical pathways sustaining peculiar physiological metabolic capabilities makes the extremophilic microbial communities of Pisciarelli interesting from an astrobiological point of view.

Conclusion

Archaea populating hydrothermal vents are life forms that, rather surprisingly, have been discovered in our planet only recently if compared

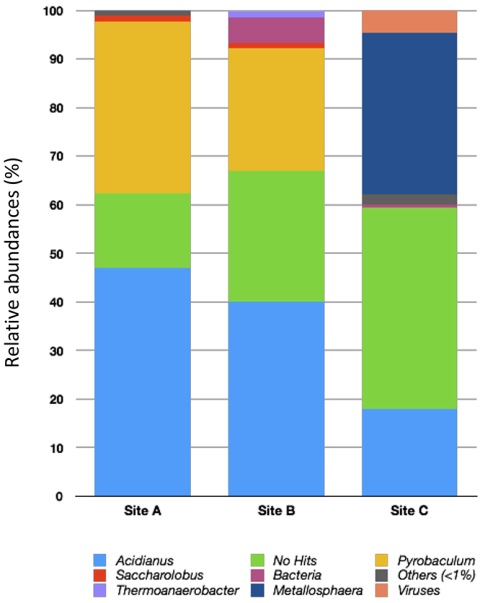

Fig. 4. Relative abundances at the genus level of the sites A, B, and C of Pisciarelli hot springs. Taxa showing less than 1% of assigned sequences are grouped as "others".

to Bacteria and Eukaryota, and are ideal model system to investigate on how cellular life appeared and evolved on Earth. Their study is improving our knowledge on several aspects that are of utmost importance in Astrobiology like defining sites in the universe that may host, or have hosted, life, searching biosignatures, and defining the first crucial molecular steps of primordial life.

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