From extreme environments to human pathogens: an evolutionary journey

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The history of our planet is underpinned by roughly 4 billion years of microbial evolution. From its emergence in a (probably) hot and anoxic environment, microbial life has evolved to colonize every available niche on our planet, including the inside and outside of other organisms. Yet, the emergence and evolution of microbial metabolism remains a major unsolved problem. How have microbes adapted to colonize every available environmental niche, including other organisms? How did they evolve to colonize mammals and our human ancestors? Answers to these questions will allow us to understand the emergence and evolution of life on our planet, inform the search for life elsewhere and, in the making, reveal important insight that will help us fight infections.

A microbial world

There is little doubt today that prokaryotes were the earliest forms of life on our planet. Life as we know it is currently based on energy-conserving reactions that allow the exploitation of naturally occurring redox gradients to perform chemical, mechanical and transport work. Over 30 years of research on the origin of life has led to recognition of the importance of molecular hydrogen, carbon monoxide and naturally occurring electron flows across mineral surfaces as possible early energy sources in protometabolism. Assuming that we have identified the earliest forms of energetic metabolism, we still have to understand how microbial metabolism diversified from relatively few early energy-conserving reactions to almost every redox couple available on earth.

On our planet, microbes influence biogeochemistry, climate and overall planet functioning. They outnumber any other living organism by far, with recent estimates of microbial biomass ranging between 9 and 31×10^{29} cells, and control all major biogeochemical cycling of elements. Microorganisms produce about 50% of the oxygen we breathe, and control the emission of important greenhouse gases like nitrous oxide and methane. They are responsible for the re-mineralization of organic matter, effectively recycling living biomass. Over time, they have influenced the overall redox state of the surface of our planet and permanently bioengineered the environment.

While the influence of environmental parameters in forcing the evolution of metabolic traits was recognized a long time ago, the ability of life to influence environmental conditions has been long overlooked. Only in the last decade have we begun to appreciate how the geosphere and biosphere have co-evolved, ultimately resulting in the complex network of metabolic reactions we see today.

Besides controlling biogeochemical cycles, microbes also affect human and animal health. Microorganisms that comprise both beneficial strains and potential pathogens colonize the exposed areas of our bodies and our gastrointestinal tract. Recent studies have highlighted the importance of a balanced microbiome on the functioning of our immune systems, and the gut microbiome has been shown to directly influence brain function via the gut–brain axis. We also know that an increasing number of pathogens are developing significant resistance to current antibiotics, and the threat of infections by multidrug-resistant bacteria is rising quickly.

Understanding how microbes have adapted to colonize every available environmental niche, including the outside and inside of other organisms, may hold the clue to a more efficient fight against microbial diseases. The reconstruction of the evolutionary history of key biochemical functions is critical to understanding this process.

In the broader context of the earth's microbiome, only very few species of bacteria are capable of causing human diseases. These pathogens evolved the ability to colonize humans in relatively recent times, possibly from environmental relatives that pre-dated humans and mammals. Phylogenetic studies suggest that some of these pathogens might have evolved from ancestors that inhabited geothermal environments. Hence, the microbiology of extreme environments may provide a new angle in understanding the emergence of pathogenesis, the role of adaptation and innovation in colonizing new

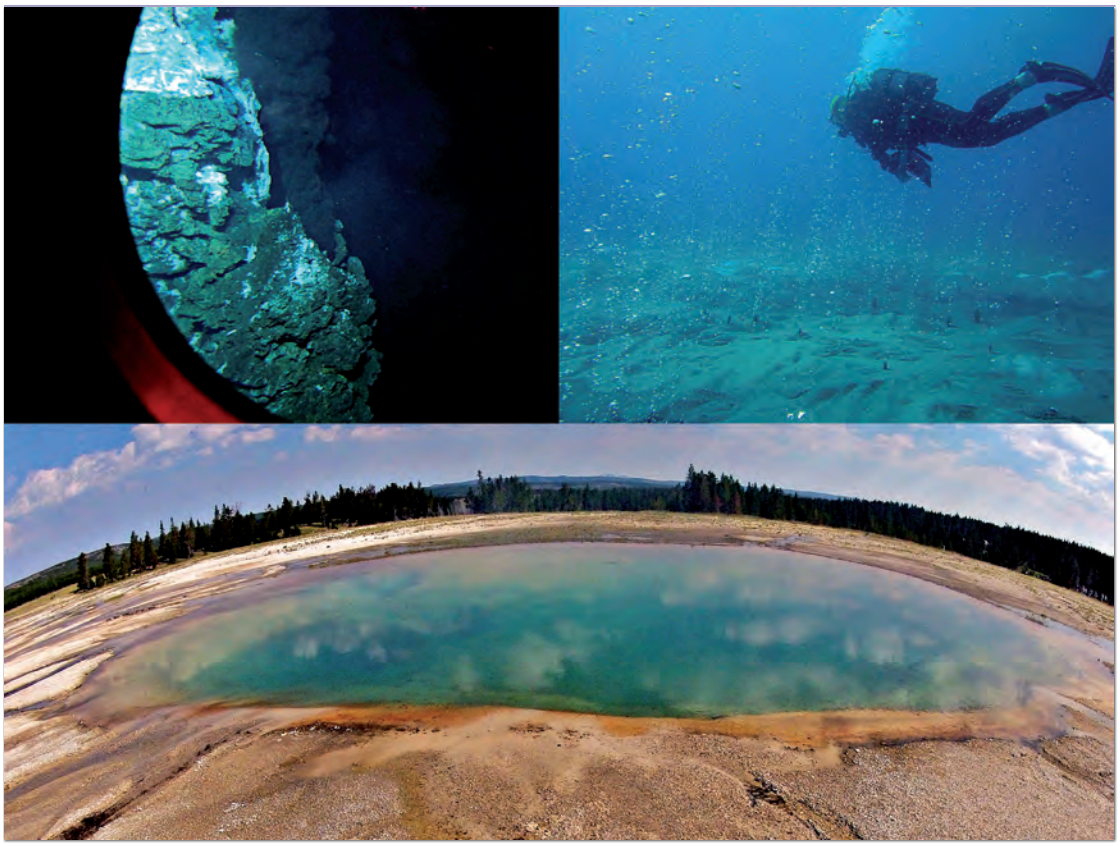

Figure 1. Example of marine and continental geothermal environments. A view of a black smoker in a deep-sea hydrothermal vent along the 9.50 °N segment of the East Pacific Rise (top left); a scuba diver swimming through the gas hydrothermal emission of the shallow-water hydrothermal vents off the coast of Milos Island, Greece (top right); and a hot geothermal pool in Yellowstone National Park, USA (bottom). Microorganisms inhabiting 'relic' environments resembling early earth, such as geothermal habitats, provide excellent models to reconstruct the emergence and evolution of metabolism.

environments, and the ecological dynamics within microbial communities. All this information may prove critical in our fight against diseases.

Extreme environments as a window into the past

Extreme environments are ubiquitous on our planet and include diverse ecosystems, characterized by a broad range of environmental conditions, e.g. high and low pH, extremes of temperatures, high salinity and pressure, low energy availability and low water activity. In particular, geothermal environments are widely distributed on our planet, both underwater and on continental settings (Figure 1). Given their relationship with volcanic activity and plate tectonics, it is safe to assume that geothermally influenced ecosystems existed throughout the entire 4.5 billion years of history of our planet, and were likely more widespread during the early stage of the evolution of our planet.

Conditions in modern geothermal environments resemble those present on the early Earth and support a broad diversity of microbial species. Therefore, microbes living in these ecosystems probably carry both ancestral metabolic traits inherited from long gone ancestors, as well as more recently acquired traits that reflect their adaptations to Earth's changing conditions. For instance, extant strict anaerobes inhabiting anoxic geothermal environments inherited the metabolic machinery to conserve energy using redox couples of volcanic origin (e.g. hydrogen and sulfur) and to fix carbon dioxide of magmatic origin. However, it appears that the same organisms also acquired the ability to cope with reactive oxygen species to adapt to the increasing atmospheric oxygen partial pressures that have arisen on earth over the last 700 million years. By contrast, it is reasonable to hypothesize that beneficial and pathogenic microorganisms that today colonize the inside and outside of higher organisms, including ourselves, have evolved recently. Complex animals emerged around 555 million years ago, mammals have been around for about 220 million years and our human ancestors for about 200 thousand years. Thus, geothermal ecosystems may be used as a natural laboratory to understand the evolutionary history of microorganisms that originated in these habitats and later adapted to colonize mammalian hosts.

Extremophiles: modern organisms, ancient trade

While extant extremophiles inhabiting geothermally influenced ecosystems are modern organisms that co-evolved with our planet and had to adapt to changing conditions, they still harbour ancestral metabolic traits. However, the process of reconstructing the emergence and evolution of metabolism is hindered by the difficulties of unequivocally distinguishing new adaptations of older functions from true innovation. Closely related microorganisms whose members inhabit 'relic' environments, such as geothermal habitats, as well as 'recent' environments, such as the gastrointestinal tract of mammals, provide excellent models to reconstruct how metabolism evolved within the various lineages.

Epsilonproteobacteria are such a group of closely related bacteria, in that they comprise organisms adapted to sulfidic environments (e.g. thermophiles from hydrothermal vents), as well as species that colonize invertebrate and mammalian hosts, including human pathogens (Figure 2). This group of bacteria includes, among numerous environmental strains, the pathogenic *Campylobacter* spp. and *Helicobacter pylori*, well-known human pathogens with relatives in all known mammals, as well as the family *Nautiliaceae*, exclusively harbouring thermophilic strains from geothermal environments. Geothermal processes have always existed on earth, well before oxygen became available as a terminal electron acceptor for respiration and facilitated the subsequent evolution of higher forms of life, all the way to mammals. Therefore, it is reasonable to hypothesize that Epsilonproteobacteria that colonize mammalian hosts, such as the rumen commensal *Wolinella*, and the human pathogens *Campylobacter* and *Helicobacter* spp., have evolved from anaerobic, thermophilic ancestors whose extant relatives are represented by species that inhabit geothermal environments (e.g. *Caminibacter*, *Nautilia*, *Cetia* spp.). Recent phylogenetic studies confirmed this hypothesis and showed that extant thermophilic Epsilonproteobacteria from deep-sea hydrothermal vents represent the most ancient lineages of this class and that their commensal and pathogenic relatives evolved more recently. Therefore, disentangling the evolutionary relationships between hydrothermal vent Epsilonproteobacteria and human pathogens can provide insight into the evolutionary history of microbial adaptation and the origin of pathogenesis.

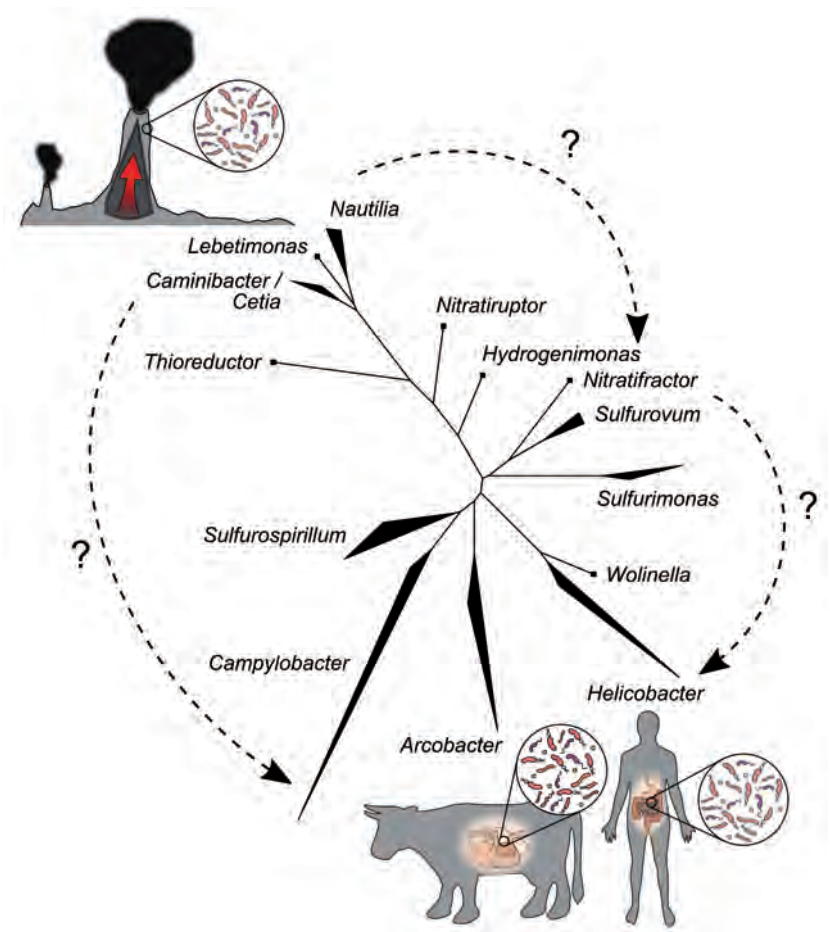

Figure 2. Phylogenetic tree showing the position of cultured pathogenic and environmental strains of the Epsilonproteobacteria. Two hypothetical patterns of gene flow from thermophilic geothermal strains (top left) to commensal and pathogenic strains (bottom) are shown.

Recently, our understanding of the ecology, physiology and metabolism of Epsilonproteobacteria has increased rapidly, and the genomes of representative members of this class have been sequenced and are publicly available. Within the Epsilonproteobacteria, it is possible to identify the 'core' genome common to all representatives of the group, the 'shell' genome, which includes all the genes shared within a subgroup of organisms, and represents common adaptive strategies, and the 'cloud' genome, which includes lost or acquired genes. Further, studies of the transcriptome and proteome of the pathogenic Epsilonproteobacteria, *Campylobacter jejuni* and *H. pylori*, have shown that when these pathogens establish an infection cycle, they develop biofilms, which are associated with the expression of genes involved in quorum sensing, flagellar motility, adhesion, exopolysaccharide synthesis and protein glycosylation, among others. Deep-sea and shallow-water hydrothermal vent Epsilonproteobacteria also establish biofilms when they colonize the oceanic crust in proximity to vent emissions or when they establish symbiotic relationships with vent invertebrates (Figure 3). The quorum sensing mechanism in pathogenic and commensal Epsilonproteobacteria, which regulated the expression of biofilm-related genes, is conserved in their deep-sea vent relatives. Understanding which portion of the pathogens' metabolic machinery has been conserved

and adapted to new conditions and what portion of their genomes is an acquired trait is fundamental in delineating the evolutionary journey from environmental strains to human pathogens.

Making of a pathogen

Numerous key metabolic functions seem to have been repurposed in the Epsilonproteobacteria lineage during the change from inhabiting extreme environments to becoming human pathogens. In the last few years, several studies have focused on the general make-up of the genomes of Epsilonproteobacteria and specific functions such as nitrate reduction, quorum sensing and chemotaxis.

Chemotaxis is a key mechanism that allows bacteria to move in response to a chemical stimulus and is important in the onset of colonization of the gastric epithelium by the pathogenic bacterium, *H. pylori*. Previous studies have identified that the ChePep protein has a key role in controlling chemotaxis in this bacterium. This peptide controls flagellar rotation and is necessary to establish *H. pylori* colonization of the gastric glands. ChePep is found exclusively in Epsilonproteobacteria and is conserved throughout this group. Genetic analysis coupled to differential interference contrast imaging of swimming behaviour and animal infections showed that *H. pylori* ChePep mutants swim abnormally and fail to colonize the gastric glands. Further, complementation of *H. pylori* ChePep mutants with the *ChePep* gene homologues from the deep-sea vent Epsilonproteobacterium, *Caminibacter mediatlanticus*, and from the zoonotic pathogen, *C. jejuni*, showed that *ChePep* is functionally conserved across the Epsilonproteobacteria. Overall, these findings indicate that the *ChePep* gene is part of the core Epsilonproteobacterial genome and may represent an early evolutionary invention that contributes to the colonization of the host, being either a deep-sea vent invertebrate or a mammalian host.

Quorum sensing is a communication mechanism between cells that depends on their density and the production of signalling molecules, and it is used by bacteria to control gene expression, including virulence genes and functions associated with biofilm formation and host colonization. One quorum sensing system that appears to be widespread across the bacterial domain is based on a furanone derivative known as autoinducer-2 (AI-2), which is synthesized by the LuxS enzyme. In 2015, Pérez-Rodríguez et al. demonstrated that LuxS is conserved in all Epsilonproteobacteria and that the mesophilic strains, including pathogens, shared a common LuxS ancestor nested within the thermophilic lineage. These findings suggest that the epsilonproteobacterial LuxS lineage originated in geothermal environments.

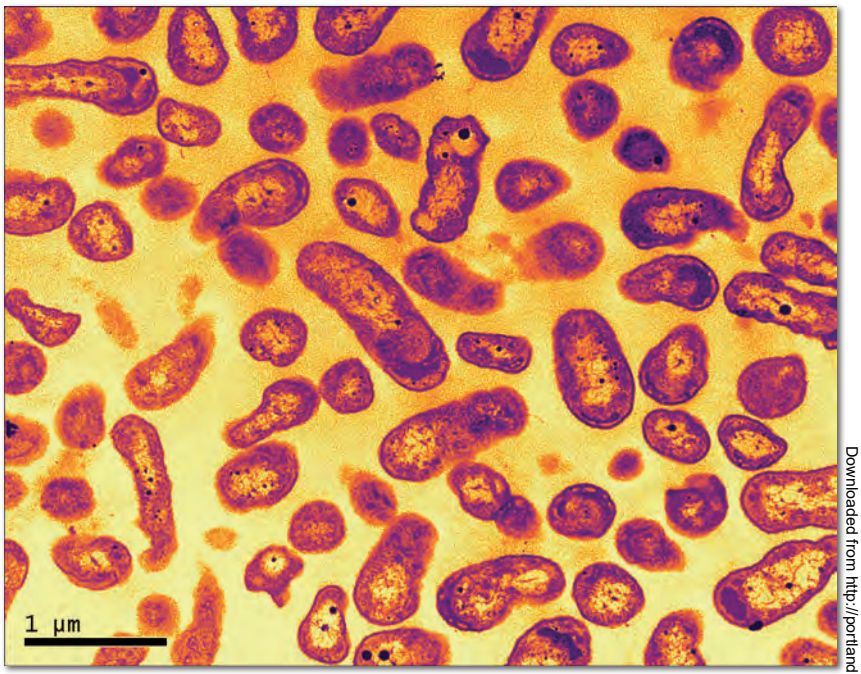

Figure 3. False color transmission electron micrograph of a pure culture of the mesophilic epsilonproteobacterium, *Sulfurovum riftiae* isolated from a biofilm growing on the tube of the deep-sea hydrothermal vent tubeworm *Riftia pachyptila*. The working hypothesis is that deep-sea vent Epsilonproteobacteria, such as *S. riftiae*, and its commensal and pathogenic relatives, adopt conserved mechanisms to colonize vent invertebrates, mammals and human hosts, respectively.

Nitrate reduction is a major pathway of respiration in both environmental and pathogenic Epsilonproteobacteria. Thermophilic members of the *Nautiliaceae* couple hydrogen oxidation to the reduction of nitrate to ammonia or to the reduction of elemental sulfur to hydrogen sulfide. This latter pathway appears to be ancient, and is widespread among anaerobic bacteria inhabiting geothermal environments. Nitrate is depleted in hydrothermal fluids, but in modern oceans it is present at millimolar concentrations in deep seawater. Vent microorganisms are exposed to it along redox gradients that form at the interface between fluids and seawater. Similar nitrate concentrations are also common in human body fluids, and nitrate has been shown to be an important signalling molecule during inflammation and at the onset of virulence in several opportunistic human pathogens. The ability to respire nitrate via the periplasmic nitrate reductase (Nap) appears to be conserved within the Epsilonproteobacteria (with the exception of some strains of *Helicobacter*) and probably represent a trait acquired during the evolutionary history of the group. Comparative analysis of the nap gene clusters of various bacteria revealed that Epsilonproteobacteria lack the NapC subunit, identified as a key component of the electron transport chain during nitrate reductase in *Gammaproteobacteria* (Figure 4). A recently formulated hypothesis posits that the Epsilonproteobacterial nap cluster could be better adapted to the nitrate-limiting conditions of both deep-sea vents and human fluids. However, this hypothesis has not been tested experimentally.

Taken together, these findings highlight the evolutionary link between environmental strains and pathogens, and underline the importance of studying the evolution and adaptation of microbial metabolisms using a broad evolutionary approach. However, growing biases in our sequence databases and a lack of functional information on key metabolic pathways of relevant organisms may hinder our ability to reconstruct the evolutionary history of key biochemical functions.

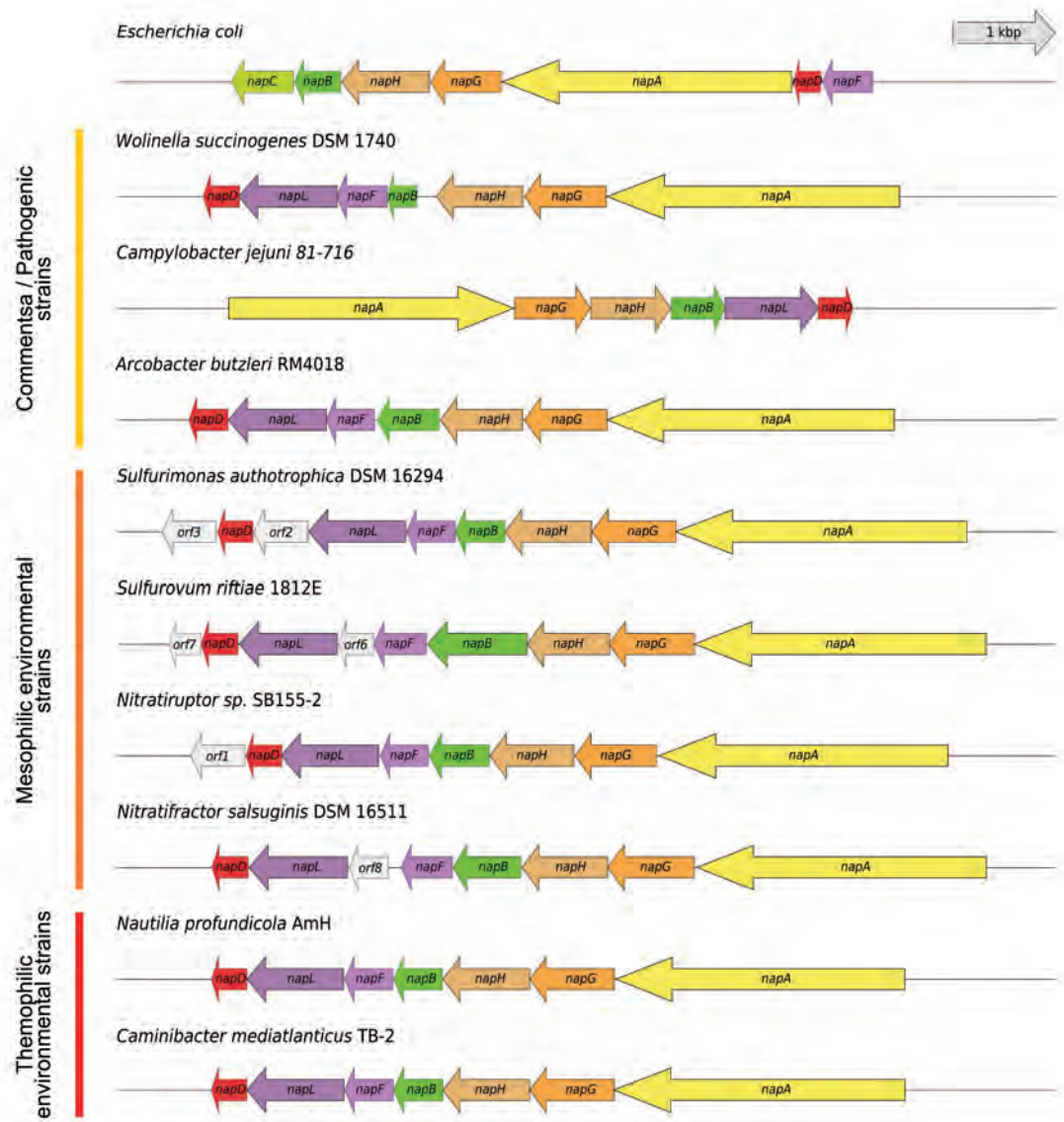

Figure 4. The conserved periplasmic nitrate reductase (Nap) operon structure in pathogenic and environmental *Epsilonproteobacteria*. The ability to respire nitrate using high affinity enzymes such as the periplasmic nitrate reductase appears to be a conserved strategy in colonizing deep-sea vents and the human gastrointestinal tract. The *nap* cluster in *Escherichia coli* is included as a reference.

Making sense of data: a problem of database quality

While there is growing excitement in the field of extremophile molecular biology, and geothermally influenced ecosystems offer an ideal laboratory for the study of the plasticity and evolution of microbial metabolism, the community is facing increasing challenges due to difficulties in annotating gene functions. The size of the sequence database is increasing exponentially, with a large number of new genomes sequenced every year, expanding the diversity of known genes. Currently, during genome annotation, function is assigned to a new gene based on sequence similarity to other genes in the database. While this approach has proved useful in speeding up the annotation of newly sequenced genomes, it is prone to error. There is a growing need for biochemical and biophysical studies on a wider set of microbial proteins from a broader range of bacteria. To properly define gene function, one needs to obtain information on the enzyme the gene encodes, including, among other parameters, substrate specificity and affinity and reaction kinetics. Such biochemical characteristics will help to constrain microbial physiology and function and reconstruct the evolutionary history of key biochemical pathways.

There are several reasons associated with the slow progress in annotating gene functions in microorganisms from geothermal environments. First, omics-based

surveys of microorganisms inhabiting geothermal environments often advance at a faster pace than the isolation of representative microorganisms necessary for physiological, biochemical and genetic studies. Second, in most cases, a genetic system that allows the generation of knockout mutants in these bacteria has not been developed. Third, the production of large quantities of purified enzymes for biochemical studies is often a limiting step with anaerobic bacteria.

These are exciting times in which the boundaries between medical microbiology and environmental microbiology are becoming blurred, and there are plenty of opportunities to unearth fundamental mechanisms that will allow us to better understand the emergence and evolution of microbial metabolism, as well as how microbes adapted to colonize every available niche on our planet. To capitalize on these opportunities, we will need a better integration of disciplines spanning from geology to biochemistry. ■

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