Life in the deep subsurface

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Life extends far deeper into the Earth’s subsurface than presumed possible 30 years ago. In the past, it was assumed that life is a surface phenomenon, and that even ‘hardy prokaryotic types’ are not capable of living deeper than tens of meters below the surface (e.g., Jannasch et al., 1971). In the 1990s, it became apparent that genetically and metabolically diverse microbial communities existed under highly reducing conditions in the deep subsurface (e.g., Parkes et al., 1994). Today we know that life in the deep subsurface is ubiquitous and comprises a large proportion of the biomass on Earth (e.g., Fry et al., 2008). However, many questions concerning life in the deep remain unanswered; for example: What is the lower depth limit of the deep biosphere? Which energy sources (i.e., electron donors and acceptors) are fueling these communities? How are genetic diversity and functional activity linked to geochemical and geological factors? What we know is that the deep subsurface is an extreme environment and that the microorganisms living here have developed numerous mechanisms to deal with high pressure and temperature, limited energy and nutrient availability, extreme acidity and alkalinity, metal toxicity, and radioactivity (Pikuta et al., 2007). The more we study extreme ecosystems the more it becomes apparent that microbial communities not only adapt to these conditions, but also change these environments to suit their ‘lifestyle.’ The study by Parkes et al. (2011, p. 219 in this issue of Geology) is a prime example of such mechanisms at work.

Organic matter trapped in sediments aside, H₂ appears to be the most important energy source available to deep subsurface communities (e.g., Nealson et al., 2005). In some subsurface environments, primary production driven by H₂, rather than photosynthesis or products thereof, is thought to fuel the entire ecosystem (Nealson et al., 2005). Previously, researchers had assumed that the formation of H₂ in the subsurface was a result of abiogenic processes. The study by Parkes et al. initiates a paradigm shift by demonstrating that subsurface microbiota are capable of ensuring a continuous supply of a primary energy source by mediating the formation of H₂ from minerals. Hence, they have a greater influence on the continued existence of deep ecosystems than previously thought. Another study has shown that bacteria possess a rather elegant system of getting rid of ‘used-up’ electrons, preventing cell death by suffocation (El-Naggar et al., 2010). The team around Y.A. Gorby (at the J. Craig Venter Institute, California) has shown that metal-reducing bacteria, in the absence of readily available intracellular electron acceptors, grow electrically conductive nanowires, which they use to transfer electrons directly onto minerals, e.g., iron oxides (El-Naggar et al., 2010). This led to the hypothesis that nanowires are also used to transfer electrons between cells in nanogrids, hence giving cells in highly reducing environments access to external electron acceptors. This may be particularly important for microbes in the deep subsurface, where dissolved terminal electron acceptors, such as O₂ or oxidized forms of sulfur and nitrogen, are in short supply, but where suitable minerals occur. Another study has shown that arsenic can replace phosphorus in the DNA, RNA, and proteins of GFAJ-1, a bacterium isolated from the hypersaline waters of Mono Lake (California), hence substituting one of the six essential elements of life (carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur) with an element widely available in the subsurface (Wolfe-Simon et al., 2010). This suggests that life may not be as elementally limited as postulated, and that a biosphere of arsenic-utilizing life may exist in the subsurface on Earth, other planets, moons, or asteroids. Despite these results, which question and extend our fundamental understanding of life, its constituents, evolution, and metabolic functions, some question why we should invest millions of research dollars into studying extreme environments deep underground; yet, studying these systems has many practical applications. We need to know how subsurface microbial processes affect the mobility of radioactive isotopes from buried atomic waste, and how microbial processes affect the underground storage of CO₂. There are also technological opportunities hidden in the deep subsurface. Subsurface microbiota may be useful for the in situ processing of underground ore deposits, cleaning up of polluted groundwaters, and, in the spirit of Parkes et al., we may be able to use the microbially mediated formation of H₂ (or other gases) as a way of producing clean energy. Paving the way for these discoveries are advances in deep-drilling technology, field-sterile sampling, microanalyses, metagenomics, transcriptomics, proteomics, and bioinformatics. These will enable geomicrobiologists to study microbial diversities, capabilities, and activities on the scale of entire subsurface ecosystems, and link microbial factors to geochemical and geological factors.

Geomicrobiology is a field of research at the interface between earth and life sciences. Its subject is the study of the influences of microbiota on geological and geochemical processes during Earth’s history. Ever since the emergence of the first microbial life on Earth at ca. 3.8 Ga, microbes have played a fundamental role as geological agents in mineral growth and dissolution; rock and mineral weathering and alteration; mobilization and precipitation of metals and formation of ore deposits; metabolism of hydrocarbons and transformation of organic carbon in sediments for fossil fuel formation; cycling of carbon, nitrogen, sulfur, and most other elements in global cycles; fractionation of isotopes; porosity generation in the deep subsurface; and the emergence of the aerobic biosphere (Ehrlich, 2002). The success of Bacteria and Archaea is largely dependent on their ecological and metabolic capabilities, which lets them thrive in extreme environments (Pikuta et al., 2007). Extremophiles are capable of growing at temperatures from −10 °C to 121 °C, at pH from below 0 to 13, under hypersaline and extremely dry conditions, at pressures above 100 MPa, at heavy metal ion concentrations of tens of millimoles, and under radiation dosages 3,000 times higher than lethal to humans (Pikuta et al., 2007). They are capable of utilizing numerous electron dons and acceptors to obtain metabolic energy, and, depending on environmental conditions, are capable of switching between metabolic pathways (Ehrlich, 2002). According to Whitman et al. (1998), between 75 and 94% of the Earth’s prokaryotes reside in the deep subsurface. Other authors suggest that these estimates are inflated due to a bias in the selection of sampling sites toward high-productivity areas, e.g., hydrothermal areas (D’Hondt et al., 2009). D’Hondt et al. (2009) have found that cell numbers and activity in sub-seafloor sediments away from hydrothermal hotspots and in the continental subsurface are orders of magnitude lower. It is my opinion that it is currently impossible to present reliable numbers concerning the deep subsurface biomass, as well as the lower limits of the deep biosphere. This is mostly due to the lack of representative drill cores covering the diverse marine and continental subsurface.

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environments. However, in recent years researchers have added substantially to our understanding of the genetic and metabolic diversity in the deep subsurface, and of how these communities are supplied with energy. Phototrophically derived organic matter in marine sediments constitutes the largest, but highly recalcitrant, pool of organic carbon on Earth, and has long been recognized as one of the main sources of metabolic energy in marine subsurface systems (e.g., Adhikari and Kallmeyer, 2010). Another widely debated source of metabolic energy is H$_2$. Abiogenic processes, such as reactions between dissolved gases in magma; decomposition of CH$_4$ to graphite and H$_2$; radiolysis of water by radioactive U, Th, and K isotopes; cataclasis of silicates under stress in the presence of water; and hydrolysis by ferrous minerals are thought to produce the H$_2$ that fuels the deep subsurface (e.g., Nealson et al., 2005). Studies suggest that chemolithoautotrophs were the first primary producers, and that the ‘tree’ of terrestrial life roots in non-photosynthetic H$_2$-driven communities (e.g., Nealson et al., 2005). Hydrogen-driven communities are said to persist in the deep biosphere until this day, and are seen as proxies for potential H$_2$-driven extraterrestrial biological systems (e.g., Nealson et al., 2005). However, other studies suggest that not enough H$_2$ can be produced abiogenically to ensure a steady supply and support H$_2$-driven ecosystems over geological periods (e.g., Anderson et al., 1998). In this context, the paper by Parkes et al. points to a biogenic mechanisms of ensuring a steady supply of H$_2$. In sediment slurry experiments (0–100 °C) with a range of common minerals, significant H$_2$ formation was observed only in the presence of prokaryotes (Parkes et al., 2011). This supports the argument that H$_2$-driven communities exist that provide the ‘base load’ for further microbial activities, e.g., sulfate reduction or methanogenesis. 

However, while trying to assess and integrate microbial diversity, functions, activities and geochemical data, many discrepancies have become apparent. For instance, the deep sub-seafloor harbors phylogenetically highly diverse communities of Bacteria and Archaea. Yet which domain is more numerous or diverse is unclear given the scarcity of samples studied, and the difficulty in correlating studies conducted using methodologies that need to be standardized. Studies based on quantitative polymerase chain reaction (PCR) on DNA extracts from deep sub-seafloor drill holes at the Peru Margin have shown that Bacteria were 10–1000 × more abundant than Archaea (Schippers and Neretin, 2006). In contrast, microscopic analyses of the same samples have shown that Archaea are more abundant than Bacteria (Biddle et al., 2006). When measured directly or inferred from geochemical data, methanogenesis and sulfate reduction are key processes in marine subsurface systems (Fry et al., 2008). However, the number of phylotypes in gene libraries related to known sulfate-reducing bacteria (SRB) or methanogens is often very small (Fry et al., 2008). This suggests that sulfate reduction and methanogenesis are either conducted by a small yet active part of the microbial communities, or that we do not know what large parts of the communities are capable of, because most of the identified phylotypes have no cultured relatives. To fill these gaps, and successfully link microbial diversities with functions and geochemical and geological factors, more deep drilling has to be undertaken. We also need to optimize and standardize metagenomic techniques for the study of subsurface systems, and integrate molecular techniques with culturing, microanalyses, and biochemical modeling. The use of standardized high-density functional and phylogenetic microarrays, e.g., GeoChip or PhyloChip, may be one way forward to understand the full biological and genetic complexity of these systems (He et al., 2007; Rastogi et al., 2010). Hence, we hope that, in the future, an integrated approach will provide further insights into the physical and chemical interactions between microbes and minerals, and how these interactions shape global element cycles and, therefore, our climate. With such a vast territory still to be explored, who knows what we will find, what is out there, or more fittingly, down there?

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