New Horizons for Deep Subsurface Microbiology

Subsurface microorganisms may grow slowly, but their diversity and persistence offer insights into life in extremis

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The U.S. Department of Energy (DOE) launched the modern era of subsurface microbiology at its Savannah River Plant (SRP) in South Carolina in 1986. Those first efforts, involving three 200-m-deep wells along with procedures to monitor for drilling-related contaminants, uncovered abundant and diverse microbial communities in subsurface aquifers (Fig. 1). Geomicrobiologists soon had seven field campaigns going, through which they explored a wide range of environments and rock types, taking core samples with a variety of fluids and tracers. Moreover, by piggybacking onto petroleum exploratory drilling programs, they soon were retrieving microbial samples from depths as great as 2.7 km. The research teams were and remain interdisciplinary, including microbiologists, geochemists, geologists, and hydrologists, drawn from U.S. universities as well as DOE laboratories. This quest quickly expanded globally with research groups in Europe and elsewhere, including the University of Bristol in the U.K., Gothenburg University in Sweden, the Environmental Institute in Denmark, and at several institutions in Russia and Japan.

By 1998, leaders in the young field of geomicrobiology agreed that a substantial fraction of living carbon biomass consists of microorganisms residing beneath the seafloor and soil zones. Moreover, biodiversity within the subsurface was striking, yielding many novel species of Firmicutes as well as some novel archaeal species. The sheer numbers of species were surprising in the face of scant nutrient fluxes. For instance, we estimated subsurface bacterial cell turnover times in terms of decades or centuries for species recovered from aquifers 200 to 400 m beneath the surface. Despite this seeming lethargy, however, the subsurface biosphere controls water chemistry and influences mineral and organic changes in sedimentary rocks.

Geomicrobiologists Probed Great Depths and Extreme Environments

Over the past decade, geomicrobiologists began probing the subsurface biosphere to depths greater than 3 km in South African gold mines, at 120°C sites in China, in ice sheets at −20°C about 1 km below the surface, beneath 0.5 km of permafrost, and within and beneath gas hydrate deposits of varying depths. Deep, hot spreading centers containing marine chemoautotrophic eco-

Summary

- A substantial fraction of living planetary biomass consists of highly diverse microorganisms residing beneath the seafloor and soil zones.
- Microbial life in subsurface zones persists in extreme environments where nutrients may be scarce, the pace of life slow, and the demand on DNA repair mechanisms intense.
- Key questions include how the same microbial species can occupy very distant subsurface sites and what are the temperature extremes at which life cannot exist.
- Experiments being designed for the Deep Underground Science and Engineering Laboratory promise insights into many subsurface microbial secrets, perhaps including clues to the origins of life.
systems contrast with cooler, subseafloor sediments, which provide glimpses of heterotrophic ecosystems living on old, buried photosynthetic materials.

In contrast to the marine realm, the terrestrial subsurface contains ecosystems whose chemoheterotrophic nature increases with depth. The rock-water interactions that fuel these continental subsurface ecosystems depend upon regional, topographically driven meteoric fluid flow. For instance, in the elevated plateaus comprising the Witwatersrand Basin and the Columbia River basaltic aquifer, groundwater can penetrate to depths of several kilometers. In the granitic aquifers of the Fennoscandian Precambrian shield beneath the Baltic Sea and in the Atlantic Coastal Plain aquifers, however, regional flow is slower and shallower.

Whether it is 1-million-year-old ice, tens-of-million-years-old saline fracture water, or Permian-age salt, in environments where fluid flux is negligible, viable cells with intact genomes survive by respiring ever so slowly, while fastidiously repairing radiation-damaged DNA. One hypothesis for this mystery is that radiolytic processes provide a renewable source of reductants and oxidants to these trapped terrestrial and marine subsurface chemoheterotrophic microbial ecosystems. A complicating factor in evaluating biodiversity is that DNA fragments of deceased microorganisms possibly formed a pool of ancient DNA, and differences found in 16S rRNA sequences from disparate sites thus might reflect evolutionary trends rather than geographical differences.

Many Remaining Microbial Mysteries of the Deep

One key mystery concerning the deep subsurface biosphere is how the same bacterial species can be found in oil reservoir fluids that are thousands of kilometers apart. This continuity may be reasonable for marine environments where cold ocean currents could distribute thermophiles, or for surface continental sites where the atmosphere is the principal transport vector. In other subsurface environments, such as shallow aquifers and seafloor spreading centers, recently termed the “subseafloor ocean,” fluid movement may also be capable of broadly disseminating microbes from a cosmopolitan source.

However, in deep subsurface environments, particularly petroleum-trapping ones, where the rock is impermeable or fluid flow is extremely slow, barriers blocking subsurface microorganisms appear to be substantial. Yet, finding the same microbial species in distant oil reservoirs implies either high microbial transport rates beneath the continental surfaces or slow transport rates combined with exceptionally slow mutation rates.

With increasing subsurface residence times, geological and climate histories may influence biogeographical distinctions in the terrestrial subsurface. If geographic isolation is important, then geochemically similar subsurface environments presumably should yield different microbial species. To address this issue, it will be helpful to obtain genome sequences of subsurface microbial species that are separated by hundreds of kilometers to determine whether genes that are present in one, but not the other, reflect their different environments. If so, were those genes acquired by horizontal gene transfer locally? Further, if low-diversity
microbial ecosystems are truly isolated from a much larger genetic pool, how do subsurface microorganisms evolve as their environments change? The discovery of viruses and CRISPR sequences in deep-fracture water environments raises the possibility that transduction may account for some genetic exchange.

What is the relationship between the planktonic and sessile microbial communities in the fractured, low-porosity rock strata that prevail in the deep-continental and marine crust (Fig. 2)? Cores from the Atlantic Coastal Plain aquifers contain higher microbial abundance and metabolic diversity than does groundwater containing only planktonic cells from the same aquifers. Surface-attached microbes from shallow, fractured-rock environments are phylogenetically distinct from the planktonic cells. Thus, variations in physical substrates provide conditions that select for specific types of microorganisms. Additionally, solid-liquid interfaces can generate steep chemical gradients, resulting in more diverse ecological niches than those offered by the bulk water phase. These factors may contribute to the generally higher diversity and higher activity in the surface-attached microbial communities than among planktonic cells.

These sessile communities are likely to be directly involved in altering and precipitating diagenetic mineral phases, and in changing the chemical and physical properties of rock. Although sampling planktonic microbial samples from vent fluids and boreholes is relatively straightforward, characterizing sessile communities from fracture surfaces has been stymied because the fracture faces are flushed with drilling fluids, which typically are heavily contaminated. New approaches are required to address this issue.

**Another Unsettled Issue: Upper Temperature Limits of Subsurface Microbes**

Another mystery concerns the maximum temperature of subsurface microbial activity. Analyses of activity in oil reservoirs indicate that 85°C appears to mark the upper temperature limit of microbial life there, even though hyperthermophiles that can live at temperatures up to 115°C were recovered from oil wells during the 1990s.

Mesocosm experiments indicate that at temperatures up to 90°C, acetogens rather than thermogenesis produce acetate, and that these acetogens may be fueled by H₂ generated by oxidizing Fe²⁺ in magnetite to Fe³⁺, which, in turn, oxidizes H₂S to sulfate. This thermally catalyzed cycling of Fe and S could also stimulate autotrophic Fe³⁺- and sulfate-reducing bacteria.

In addition to molecular-stability constraints, the ultimate temperature limit for life in the subsurface depends upon the balance between maintenance energy demand rate, which appears to increase with temperature, and the energy supply rate from a mix of biochemical and purely chemical reactions. This balance dictates physicochemical limits of subsurface life and suggests that salinity, redox gradients, thermal gradients, radiation, fluid flow, and even tectonic activity contribute to sustaining life.
Does subsurface hyperthermophilicity occur only where fluid flow rates and hence nutrient supply rates are elevated? Addressing this question will require more in situ observations, including drilling into hot rock at varying depths, as well as extended-scale mesocosm heating experiments.

We need to sequence genomes of subsurface isolates as well as metagenomes and to conduct functional analyses of environmental samples from great depths, not only to learn what subsurface microorganisms are capable of doing, but what they are doing in situ. The glacial pace of in situ microbial respiration hampers microarray analyses of mRNA. Detecting such signals may require altering subsurface environments, followed by long term monitoring of community responses.

In The Deep Hot Biosphere, the late astrophysicist Thomas Gold speculated that life originated beneath the surface of the earth, a hypothesis that some geomicrobiologists are exploring as a way of understanding how life on Earth recovered from the sterilizing effects of the 3.9 Ga, late-heavy bombardment, or lunar cataclysm, on its surface. Bench-top experiments invoke geochemical conditions that could occur in a subsurface setting. Does the equivalent of Darwin’s “warm, little pond” exist in the subsurface and support prebiotic chemistry? Water vugs—some isolated for millions of years—exist, and some are associated with hydrothermal deposits. Aseptic sampling of these natural, rock-encased water-storage sites could provide data with which to evaluate parts of Gold’s hypothesis.

**Plans for Studying Microbial Life in Deep Underground Laboratories**

The International Continental Drilling Program and the Integrated Ocean Drilling Program (IODP) held independent workshops in September, both of which considered questions related to the deep biosphere. Bringing these two groups together would present new opportunities for land-sea transect expeditions and for exploring life underground.

Useful though that approach could be, analyzing core samples provides only snapshots of the microbial biosphere. A fuller analysis requires programs that cover a very broad range of space and time. For instance, the scheduled South Pacific Gyre, North Pond, and Juan de Fuca IODP drilling proposals are dedicated microbiology expeditions, and the latter two are designed to capture kilometer-scale hydrologic circulation cells to evaluate their energy and microbial inputs and outputs. These drilling projects coupled with long-term observations at borehole installations should help to delineate a 4-dimensional picture of the subseafloor ocean biosphere.

Research activities to explore deep subsurface life are coalescing. The Dark Energy Biosphere Institute (DEBI), newly funded by NSF as a research coordination network, will offer opportunities to plan expeditions to study subsurface life. DEBI workshops will focus on observatory science, microbiology of sediments and the crust, biogeochemistry, and an integrated understanding of marine and continental subsurface biological systems. The workshops all aim to bring young and established scientists together to expand ways that we study and conceptualize life in the deep earth.

The only comparable effort in the continental subsurface is the Åspö Hard Rock Laboratory below the east coast of Sweden, where Karsten Pedersen and his collaborators study microbial communities within fracture fluids. Their approach involves circulating water from boreholes into microcosms using high-pressure tubing, pumps, and anaerobic chambers. Plans for similar types of experiments are being developed for several other underground research laboratories.

Meanwhile, ambitious plans exist for the proposed Deep Underground Science and Engineering Laboratory, or DUSEL, which takes advantage of the deepest (2.4 km) and largest (more than 6 km horizontally) underground mine in the United States, formerly Homestake gold mine in the Black Hills of South Dakota (Fig. 3a). This program, to be led by Kevin Lesko, a physicist, and William Roggenthen, a geophysicist, will provide the largest and deepest laboratory for high sensitivity detectors for dark matter, nucleon decay, double β decay and a spectrum of neutrinos, including a beam of neutrinos from Fermilab, which is about 1,300 km away.

DUSEL also will provide a multilevel drilling platform for observing the ecohydrology of shallow to deep continental environments where meteoric water flow is the primary mixing
mechanism. A high-capacity filtration plant to generate water for neutrino detectors can also supply water for microbiology drilling projects, obviating the need for closed-loop mud tanks with their high concentrations of contaminants. Another advantage of underground coring compared to drilling from the surface is that drilling water can be circulated down the outside of the coring barrel and up the center. Thus, when the coring bit intersects a water-filled fracture at pressure, the core is ejected with little contamination of the fracture surface.

DUSEL Expected To Provide Many Microbial Insights

The DUSEL facility will have access to ground water of diverse ages, according to modeling studies (Fig. 3b). On the south side, water may flow quickly downward from the ground surface to reach depths of 1 km in less than 1 year. In contrast, water reaching the lower depths of the north side of the mine comes from rock pores and may be thousands or more years old. The potential capture footprint extends outward from current mine workings for kilometers and could provide access to up to 100 km³ volume for microbial biogeographic studies (purple zone, Fig. 3b).

From the existing infrastructure at the 2,438-m level where the rock temperature is 55°C, boreholes (Fig. 3b, yellow lines) can be extended downward an additional 3–4 km to reach the 120°C isotherm and to explore the upper temperature limit of life. The boreholes will be used to withdraw fluids and will become
experimental stations for conducting in situ transcriptomic and proteomic experiments, either with mobile underground laboratories (MULEs) as done at Aspö or via push-pull experiments within the packer-sealed fractures themselves.

DUSEL also offers the advantage of operating in a facility for assay and acquisition of radiopure materials (AARM) at the 1,474-m level, where background radiation is $10^{-5}$ times that of typical surface counting facilities. The AARM laboratory will facilitate the use of ultrapure radiolabeled compounds for quantifying very slow metabolic or catabolic processes during push-pull experiments or to map these processes using microautoradiographic analyses of microcosm experiments.

To date, three other microbially related facilities are proposed for DUSEL: the coupled thermal-hydrological-mechanical-chemical-biological experimental (THMCB) facility, the fracture processes facility (FPF), and the DUSEL-CO$_2$ facility. The THMCB experiments will be constructed at the 1,474-m level using borehole arrays to access intact volumes of rock. Plans call for experiments with multiyear heating and cooling cycles, with the goal of attaining 250°C at the center of the block to simulate hydrothermal conditions and induce convective circulation of fluids within fractures. Geomicrobiologists will monitor how microbial communities within fractures respond to increased thermal and fluid fluxes, changes in nutrient concentrations, and how quickly microorganisms colonize heat-sterilized zones during the cooling cycle. The temperature ranges of the THMCB experiments overlap those of the ultra-deep boreholes from the 2,438-m level and provide an experimental foundation for in situ observations.

The FPF will study the creation and reactivation of faults and fractures in rock at the 90-, 1,474-, and 2,250-m levels. Alternate heating and cooling of boreholes will expand and contract the rock mass, inducing displacements along preexisting fractures and creating new faults. Repetitive thermal cycling will lead to repetitive fracturing. Fractures created during this experiment will enhance permeability and could release H$_2$, which in turn could enhance microbial activity. The FPF is not only designed to determine what controls rock strength, but it could also determine to what extent subsurface chemosynthetic activity is regulated by tectonic events.

Although the fractured, low-porosity rock of Homestake mine would seem to preclude research into CO$_2$ sequestration, the DUSEL CO$_2$ facility will take advantage of existing, large diameter, subvertical boreholes that run from the 334- to 882-m level. They will be cased and filled with sand and clay, enabling them to operate at pressures where supercritical CO$_2$ is stable. They should provide a detailed picture of how CO$_2$ transport processes are affected by the phase transition to gas or liquid. Smaller microcosm experiments attached to the main borehole assembly will be used to investigate how microbial activity is affected during CO$_2$ injection. This facility thus provides a pore-to-kilometer-scale platform for developing models for CO$_2$ sequestration.

These experiments represent a subset of those planned for the first 5 years of DUSEL, and DUSEL is open to proposals for more experiments. With its extensive infrastructure, kilometer-scale spatial access, and a multidecade lifetime, DUSEL will usher in the next generation of deep terrestrial biosphere studies. It promises insights into many of its well-hidden microbial secrets, and perhaps the origin of life itself.

SUGGESTED READING


Lin, L. H., J. Hall, J. Lippmann-Pipke, J. A. Ward, B. S. Lollar, M. DeFlaun, R. Rothmel, D. Moser, T. M. Gihring, B.


