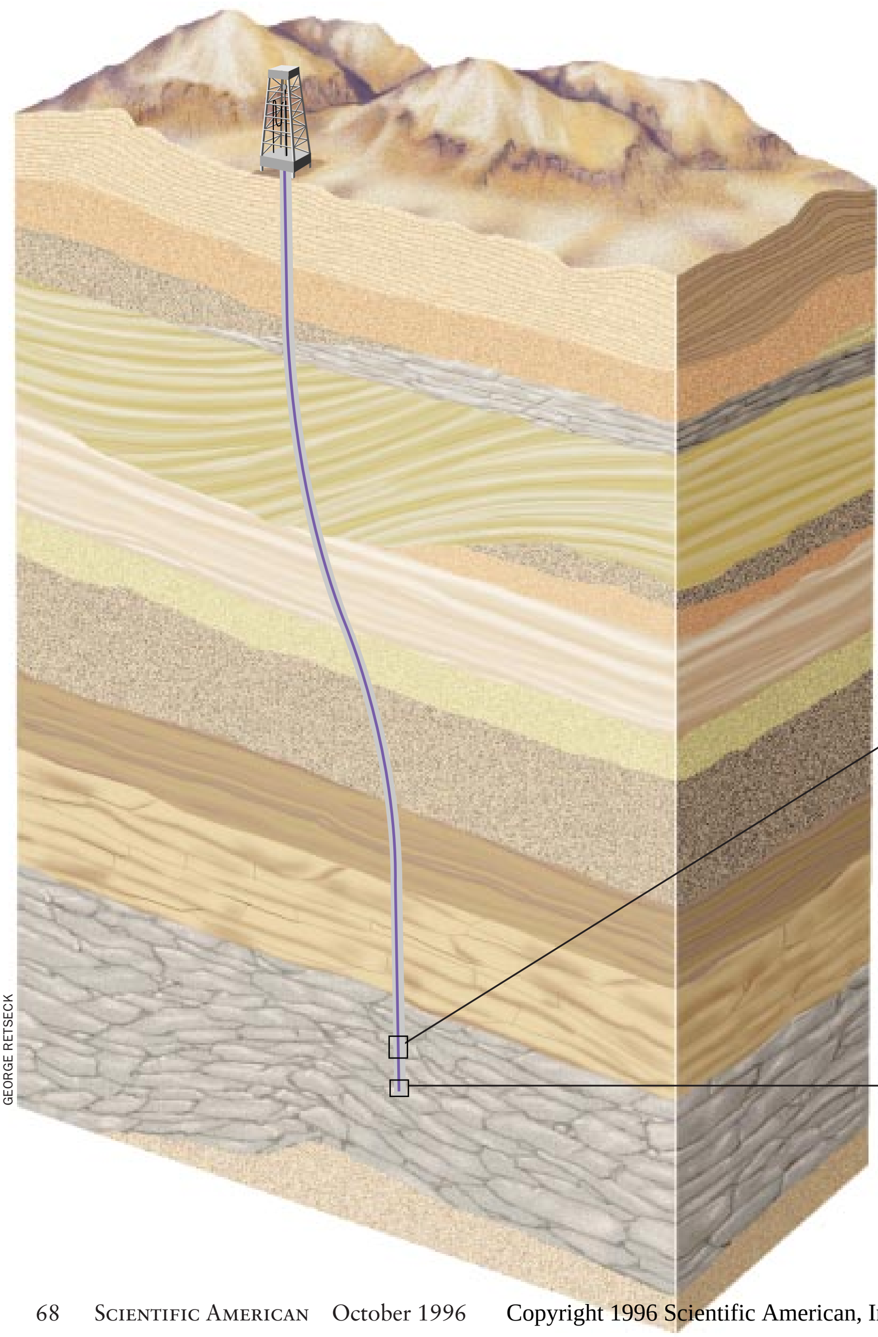
Microbes Deep inside the Earth

Recently discovered microorganisms that dwell within the earth's crust could reveal clues to the origin of life

by James K. Fredrickson and Tullis C. Onstott

Single-celled organisms—bacteria, fungi and protozoa—thrive on all parts of the earth's surface. Their habitats range from the boiling hot waters of thermal springs to the pleasantly cool soils of backyard gardens. Microorganisms provide essential services to other creatures by decomposing waste products and forming nutrients. Some microbes also inflict harm by infecting higher organisms and causing disease. Fortunately, scientists have learned to control many of those damaging effects and to expand on the ways microorganisms benefit humankind.

Although people have used the metabolic activities of microorganisms for thousands of years to produce cheese, wine and bread, it was not until the mid-20th century that scientists harnessed microbes to create antibiotics and other pharmaceuticals. Today people also employ microorganisms for such diverse tasks as controlling pests, treating sewage and degrading oil spills. With countless novel uses still awaiting discovery, biologists continue to scour the



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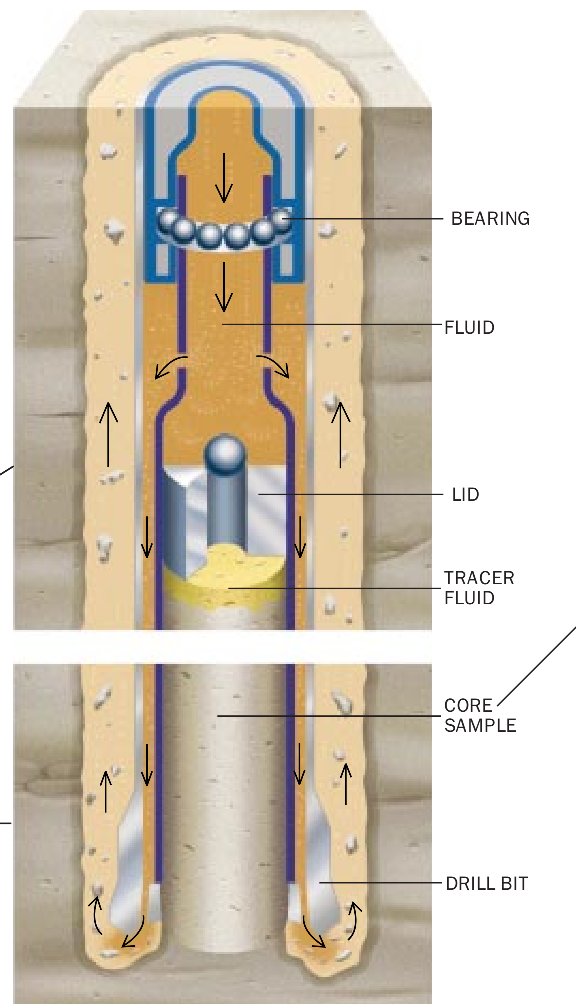


surface of the earth in search of microbes that might prove valuable in formulating new drugs or improving industrial processes. But until recently, few such bio-prospectors thought to look deep inside the earth. Long-standing scientific dogma held that this realm was essentially sterile. But that belief, as it turns out, was wrong.

It's Alive!

The first hints that microorganisms lived in the deep subsurface—hundreds to thousands of meters below ground—emerged in the 1920s from the studies of Edson S. Bastin, a geologist at the University of Chicago. Bastin questioned why water extracted from oil fields contained hydrogen sulfide and bicarbonate. After puzzling for some time, Bastin ventured an explanation. He knew that so-called sulfate-reducing bacteria can exploit sulfate for respiration in places on the surface where no oxygen is present. So Bastin reasoned that such bacteria must also live in underground oil reservoirs and produce hydrogen sulfide and bicarbonate when they degrade organic components in oil. By 1926 Bastin and Frank E. Greer, a colleague at the University of Chicago who specialized in microbiology, had succeeded in culturing sulfate-reducing bacteria from groundwater samples extracted from an oil deposit that was hundreds of meters below the surface.

Bastin and Greer speculated that these microbes might have been descendants of organisms buried more than 300 million years ago when the sediments that constituted the oil reservoir were deposited. But they had no way to test this intriguing hypothesis. At the time, many scientists viewed with skepticism the very idea of microorganisms living deep underground, noting that oil-drilling techniques were not designed to obtain samples uncontaminated by microorganisms from the surface. With little acceptance or support in the scientific community, the views of Bastin and Greer languished.

Interest in the microbiology of petroleum deposits temporarily revived during the late 1940s and 1950s, when Claude E. Zobell of the Scripps Institution of Oceanography and his colleagues investigated microbial processes in sediments buried far below the seabed. But research into subsurface microbiology again fell into dormancy during the 1960s and 1970s. Despite the importance of rock formations as reservoirs and conduits for water supplies, few considered the possibility of microbial activity deep underground. Most researchers believed that water underwent predominantly inorganic chemical alterations as it passed through the earth and that biological influences were restricted to near-surface soil layers. These scientists routinely assumed that any microbes found in groundwater samples taken from great depths were surface contaminants.

Then, during the late 1970s and early 1980s, concerns about the quality of groundwater stimulated some investigators at the U.S. Geological Survey and the Environmental Protection Agency to reevaluate their understanding of groundwater chemistry. This work spurred them to reconsider the possibility that microorganisms could inhabit water-yielding rock formations. At the same time, the U.S. Department of Energy (DOE) faced the daunting task of cleaning up the industrial facilities where nuclear materials had been produced. (As a cold war expedient, the DOE had dumped vast quantities of waste—including organic-rich solutions, metals and radioactive materials—into the subsurface at these sites.) DOE scientists were also studying how to build underground repositories that could isolate high-level radioactive wastes for thousands of years.

During this period, Frank J. Wobber, a geologist and manager at the DOE, reasoned that if microorganisms were present well below the earth's surface, they might helpfully degrade buried organic pollutants or dangerously disrupt the integrity of closed chambers containing radioactive waste. But a great deal of fundamental research needed to be done before such practical concerns could be addressed. And so he began a special effort, called the Subsurface Science Program, within the DOE. His idea was to sponsor a diverse group of biologists, geologists and chemists to search systematically for deep-seated lifeforms and examine their activities.

Because water brought up from deep drill holes is easily contaminated with organisms living near the surface, the team assembled by Wobber decided to study pieces of rock instead. But first the group needed a way to collect clean, intact samples of rock (cores) from deep in the crust.

Tommy J. Phelps of Oak Ridge National Laboratory and W. Timothy Griffin of Golder Associates rose to the challenge by designing a special drilling apparatus that minimized contact of the core samples with the drilling fluid needed to provide lubrication in a borehole. And James P. McKinley of Battelle, Pacific Northwest National Laboratory, along with F. S. (Rick) Colwell of Idaho

DAVID BOONE *Oregon Graduate Institute*

SUBSURFACE EXPLORATION (*far left*) requires a great length of rotating steel pipe to snake downward from a drilling derrick to an underground target. As the pipe rotates, a diamond-studded drill bit at the bottom of the borehole (*detail, bottom left*) cuts away at the underlying rock and surrounds a cylindrical sample that is later extracted when the pipe is withdrawn. Lubricating fluid with a special tracer substance is pumped down the center of the pipe (*detail, top left*) and out through holes in the bit (*arrows*). The cylindrical rock sample remains in place as the pipe and bit rotate because it sits within a stationary inner barrel that is supported by a bearing. As a core of rock fills the inner barrel, a bag of concentrated tracer material above it breaks open and coats the outer surface of the sample (*yellow*). Cores recovered in this way are cut into short segments from which the outer rind marked by the tracer is removed to avoid contamination (*above, left*). Within pristine inner core samples, deep-living bacteria (*above, right*) can be found.

National Engineering Laboratory, formulated special "tracers"—additives that could be mixed with the drilling fluid to indicate whether this liquid (and any microorganisms carried inside it) could have penetrated the core samples.

Striking It Rich

The search for subsurface microbes began in 1987, when the DOE arranged to drill several deep boreholes in South Carolina near the Savannah River nuclear materials processing facility. With the operators of the drilling rig there, a field team of scientists labored to avoid microbial contamination. Researchers diligently added tracers and monitored procedures around the clock as drilling proceeded. When the drillers brought a core to the surface, a member of the team quickly encapsulated the sample and placed it in a "glove bag" for processing. Those plastic containers provided a sterile environment filled with an unreactive gas (nitrogen) as a precaution to protect any so-called obligatory anaerobes—bacteria that would be quickly poisoned by the oxygen in the air.

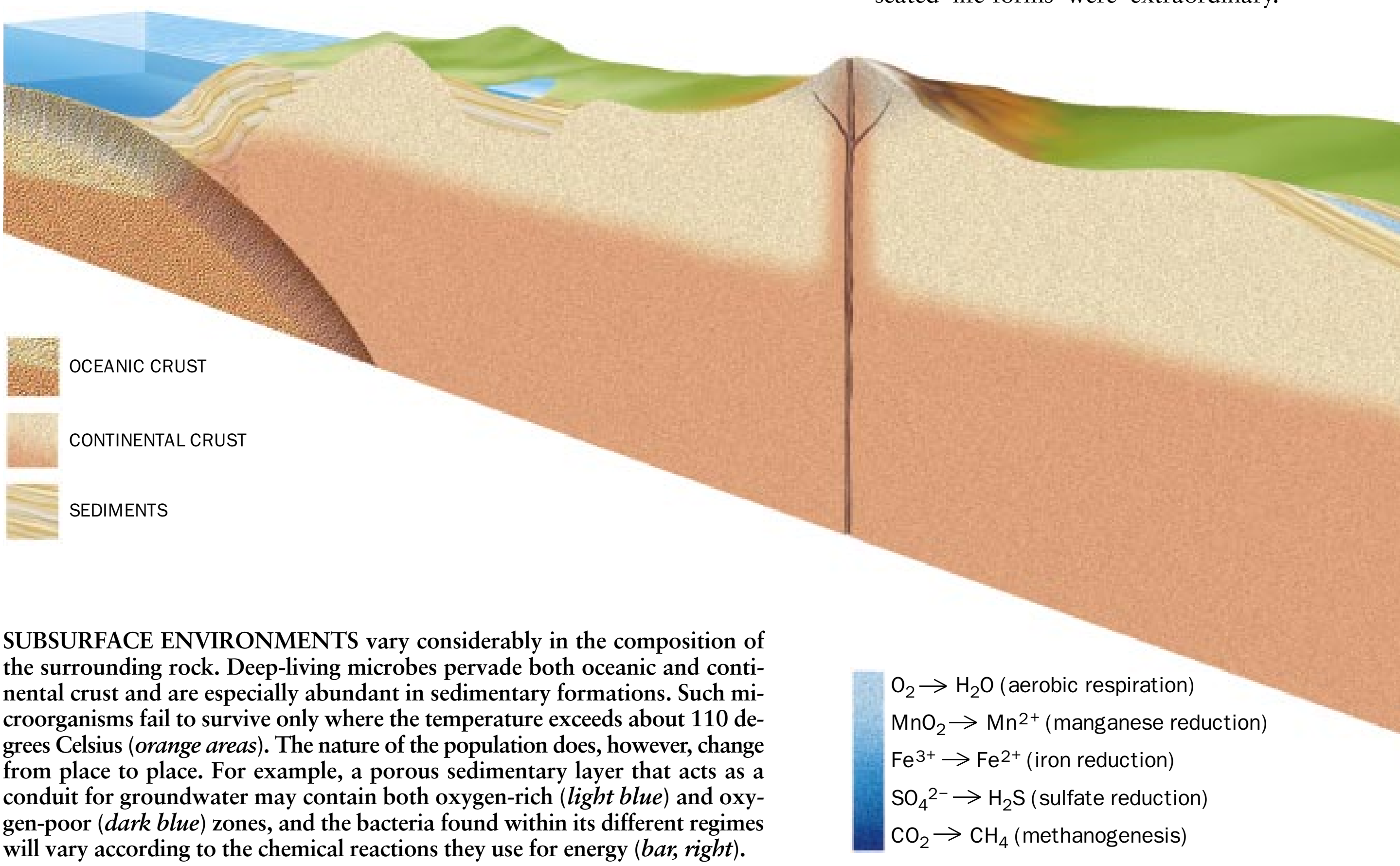
Using surgical rubber gloves attached to the interior of these bags, members of the team used sterile tools to pare away the outermost rind of each core sample, leaving only the part that was least likely to have been exposed to bacterial contaminants in the drilling fluid. If seepage of the tracer chemical indicated that a particular specimen might have been tainted, the scientist dissecting it noted that the core from which it came was very possibly contaminated.

Pristine inner core samples recovered in this way were then placed in sterile containers filled with nitrogen, which were packed in ice and shipped to research laboratories across North America. Within 72 hours after the removal of the rocks from the subsurface, other members of the research group based at many different institutions were subjecting the samples to a battery of tests designed to evaluate the rocks and the microorganisms they harbored. After these initial experiments, researchers sent the microbes they had extracted from the subsurface samples to special repositories in Florida and Oregon to be stored in liquid nitrogen at -96 degrees Celsius.

The first results of this quest for deep-seated life-forms were extraordinary.

PHIL LONG *Battelle, Pacific Northwest National Laboratory*

GLOVE BOX, with its rubber gloves protruding inward, allows scientists working near the drill sites to manipulate solid samples extracted from the subsurface. These plastic enclosures are filled with an unreactive gas to prevent oxygen from damaging delicate microbes within the recovered cores of rock.



SUBSURFACE ENVIRONMENTS vary considerably in the composition of the surrounding rock. Deep-living microbes pervade both oceanic and continental crust and are especially abundant in sedimentary formations. Such microorganisms fail to survive only where the temperature exceeds about 110 degrees Celsius (*orange areas*). The nature of the population does, however, change from place to place. For example, a porous sedimentary layer that acts as a conduit for groundwater may contain both oxygen-rich (*light blue*) and oxygen-poor (*dark blue*) zones, and the bacteria found within its different regimes will vary according to the chemical reactions they use for energy (*bar, right*).

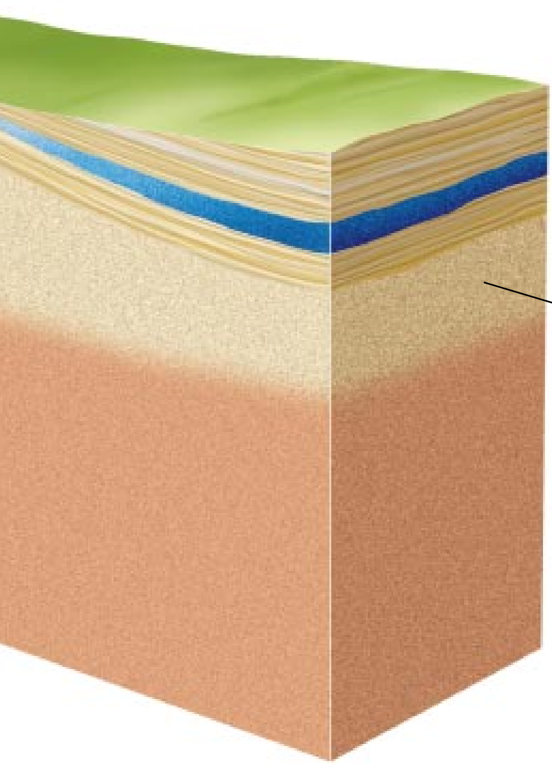
The scientists involved quickly learned that diverse types of microorganisms lived beneath the Savannah River site at depths extending at least as far as 500 meters beneath the surface, the deepest core taken. We and our many colleagues working under the aegis of the DOE's Subsurface Science Program have since examined many other geologic settings. Although we are still unsure of the extent of fungi or protozoa, the results clearly indicate that subsurface bacteria are ubiquitous. We have now recovered these organisms from formations with temperatures as high as 75 degrees C (167 degrees Fahrenheit) and from depths extending to 2.8 kilometers (1.7 miles) below the surface.

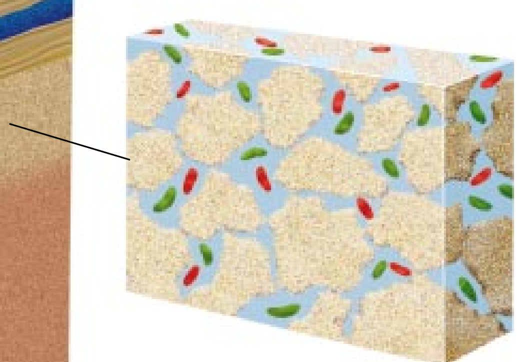
What determines the maximum depth at which subsurface microbes can exist? Mounting pressure exerts little direct effect on microorganisms even several kilometers below ground level. It is the increasing temperature that limits the depth of subsurface life. The maximum temperature that such organisms can tolerate remains something of a mystery, but biological oceanographers have found bacteria that are capable of growing at 110 degrees C in deep-sea volcanic vents, and some scientists estimate that subsurface microorganisms might be able to withstand temperatures as high as 140 degrees C, at least for short periods.

For oceanic crust, where the temperature rises about 15 degrees C per kilometer of depth, tolerance of 110 degrees allows microbial life to extend (on average) about seven kilometers below the seafloor. For continental crust, where the temperature is often near 20 degrees C at the surface and typically increases by about 25 degrees per kilometer, microscopic life should, on average, reach almost four kilometers downward into the earth.

The abundance of microbes will, however, vary considerably from place to place, even at the same depth in the earth. For example, we have discovered that samples obtained from 400 meters below the surface of the ground can contain as few as 100 to as many as 10 million bacteria in each gram of rock. John R. Parkes and his colleagues at the University of Bristol have found somewhat higher concentrations of microorganisms living in sediments beneath the ocean floor. In comparison, agricultural topsoil typically contains more than one billion bacteria in each gram of dirt.

It seems that the richness of life in the deep subsurface depends not only on tolerable temperatures but also on the capacity of the local environment to support growth and proliferation. Crucial prerequisites include the presence of water and the sheer availability of space in the pores of the rock. The region hosting the microbes must also contain the nutrients—such as carbon, nitrogen, phosphorous and various trace metals—that microorganisms need to synthesize their cellular constituents, including DNA and proteins. The environment also has to offer some form of fuel to provide the energy required for this ongoing activity.





SLIMES, or subsurface lithoautotrophic microbial ecosystems, exist in the pores between interlocking mineral grains of many igneous rocks. Autotrophic microbes (*green*) derive nutrients and energy from inorganic chemicals in their surroundings, and many other microbes (*red*), in turn, feed on organics created by autotrophs.

From Sandstone to SLiMEs

The types of microbes found in the earth's deep realms depend on the particulars of the local subsurface environment. Diverse bacterial communities thrive in most sedimentary rocks, which commonly contain a rich supply of organic compounds to nourish microorganisms. These nutrients were originally produced by plants at the earth's surface before the loose sands, silts or clays that constitute most sedimentary formations were buried and consolidated into solid rock. As long as these nutrients remain available, microorganisms living within the pores of the sediments can continue to survive and grow. Sedimentary rocks also supply oxidized forms of sulfur, iron and manganese that can provide the energy these microbes need. The chemical power sources here are so-called reduction reactions (processes that involve the gain of electrons).

As sediments become more deeply buried over geologic time, they are increasingly compacted. Much of the dwindling pore space eventually becomes cemented with minerals that precipitate from fluids passing through the rock. Consequently, as depth and pressure increase, the opportunity for obtaining life-sustaining materials declines, and the overall rate of metabolism of microbial communities gradually diminishes, except in those spots that directly surround rich concentrations of nutrients. The distribution of microorganisms in sediments ultimately becomes quite patchy. Small colonies—or even individual cells—live well separated from one another within the rock. Not surprisingly, then, searching for microorganisms living in these settings proves to be a hit-or-miss affair. Todd O. Stevens of Battelle, Pacific Northwest National Laboratory has found, for example, that with sediment collected near the DOE's Hanford facility in Washington State, the larger the sample tested, the better the chances of finding microbial activity.

Although quite inhospitable, such hardened sedimentary rock is not the most challenging environment for subsurface microbes: some environments appear far more hostile. The bulk of the continental crust is composed of igneous rock (that is, rock solidified from molten magma), which contains little organic carbon. Nevertheless, Stevens and McKinley discovered bacteria living within igneous formations that are composed of layers of basalt (a dark, fine-grained type of rock).

Microorganisms thrive in other igneous rock as well. Karsten Pedersen of the University of Göteborg in Sweden detected bacteria in water flowing through deep fractures in granite—a light-colored, coarse-grained variety of igneous rock. Because igneous rock is too hot to support life when it is first formed, the microbes found within such rock must have been carried there by the flow of groundwater sometime after the parent magma cooled and solidified.

Little buried organic matter is available within igneous formations, and so Stevens and McKinley were surprised to find that microbes could flourish in basalt. They eventually discovered the secret. The bacterial communities living there include so-called autotrophs, organisms that synthesize organic compounds (proteins, fats and other biological molecules rich in carbon) from inorganic sources. Many types of autotrophic bacteria capture energy from inorganic chemical reactions involving iron or sulfur. The autotrophs living in these basalts use hydrogen gas for energy and derive carbon from inorganic carbon dioxide. These "acetogens" then excrete simple organic compounds that other bacteria can in turn consume. In these basalts the hydrogen gas is produced by the reaction of oxygen-poor water with iron-bearing minerals. Many of us call such environments "SLiMEs," for subsurface lithoautotrophic microbial ecosystems. Amazingly, SLiME microorganisms can persist indefinitely without any supply of carbon from the surface.

Old as the Hills?

Like Bastin and Greer working decades before us, we wondered whether subsurface bacterial colonies might survive for as long as the rocks that host them. Such longevity is clearly not always possible. The continuing burial of sediments can ultimately raise temperatures sufficiently to purge an entire rock formation of live bacteria. More local sterilization may also occur where fiery hot magma impinges on sedimentary strata, leaving a body of igneous rock with some well-baked sediments surrounding it. Once such newly solidified rock cools, or tectonic forces lift hot, deeply buried sedimentary layers to a cooler position closer to the surface, bacteria carried by groundwater will then colonize the formerly sterile zones.

Yet that process of infiltration can be exceedingly slow. Ellyn M. Murphy of Battelle, Pacific Northwest National Laboratory has determined, for example, that the groundwater now present deep beneath the Savannah River facility has not been in contact with the surface for thousands of years. In the deepest sites we have examined, our measurements and computer modeling indicate that the groundwater has been isolated from the surface for millions of years. Because microorganisms could not have traveled downward from the surface faster than the groundwater descended, some subsurface microbial communities must be at least several million years old.

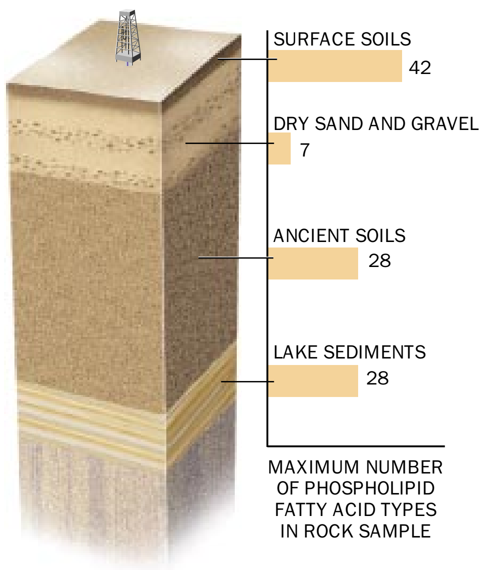
How do microorganisms manage to persevere for so long? In some cases (for example, SLiMEs), bacteria can survive because the essential nutrients are constantly renewed; although in most other sorts of formations, food and energy sources are relatively scarce. Neverthe-

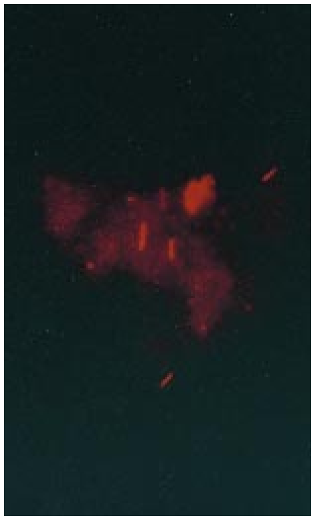
Biodiversity in the Subsurface

Just as countless kinds of life-forms cover the surface of the earth, many different types of bacteria live deep inside the crust. But because different microbes often look very much alike under the microscope, scientists have to resort to creative methods to gauge the extent of this bacterial diversity.

Certain methods allow researchers to avoid having to culture the microbes first. Biologists can, for example, apply a procedure called epifluorescence microscopy to visualize bacteria living within rock samples. This technique takes advantage of the unique makeup of the ribosomal RNA found in different types of bacteria (ribosomes are structures used by the cells to construct protein molecules). By first fashioning short strands of DNA so that they bind to particular kinds of ribosomal RNA, one can rapidly determine the variety of bacterial families in a sample. These DNA probes include a fluorescent dye so that when bacteria accumulate this substance, they seem to glow when viewed in an epifluorescence microscope (*micrograph*).

Another way to assess bacterial communities is to analyze samples for distinctive organic molecules called phospholipid fatty acids. These long carbon chains are the building blocks of bacterial cell membranes. Their molecular structure (which can be ascertained using modern laboratory instrumentation) provides a fingerprint for different bacterial families. If many different types of the fatty acid chains are found within a given sample, a diverse bacterial community exists within it. In contrast, finding a small number of distinct fatty acid molecules indicates a community of limited variety. At a site near the Department of Energy's Hanford facility in Washington State, drilling revealed striking variation in the bacterial diversity of different subsurface environments.



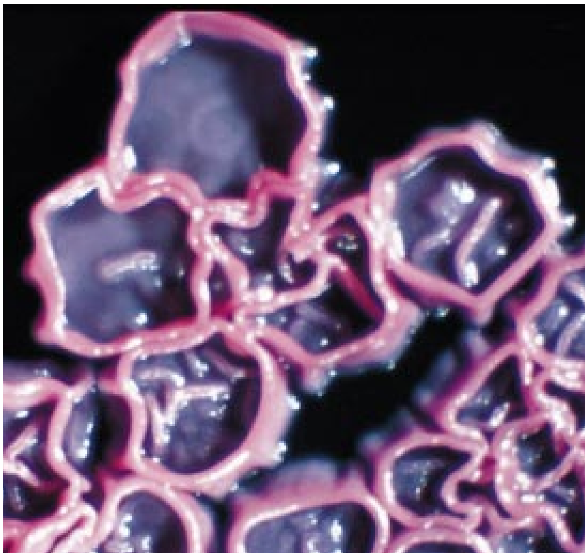


SANDRA A. NIERWICKI-BAUR *Rensselaer Polytechnic Institute* (*micrograph*); SOURCE: DAVID C. WHITE AND DAVID B. RINGELBERG *University of Tennessee*; GEORGE RETSECK (*diagram*) less, the resident bacteria appear to have adapted to these rather spartan living conditions. Bacteria must rely on internal reserves during periods of long-term starvation (as do higher organisms), and most types of bacteria shrink from a healthy size of a few microns to less than a thousandth of their normal volume as they use up their stores. Thomas L. Kieft of the New Mexico Institute of Mining and Technology has found that such tiny, starved microbes (called dwarf bacteria or "ultramicro-bacteria") commonly inhabit the subsurface.

The metabolic rate of such starved bacteria is probably much lower than when they are well fed. As a result, the average frequency of cell division for a subsurface microbe may be once a century, or even less, whereas surface microorganisms reproduce in a matter of minutes, hours, days or, at most, months. Microorganisms living in the deep subsurface limit their metabolism in order to endure starvation for geologically significant lengths of time. These bacteria can remain viable at little or no metabolic cost.

The sluggish pace of microbial metabolism in the subsurface makes it difficult to define just how many of the bacteria found entombed in these rocks are truly alive. One approach is to count only those microbes that can be grown in the laboratory. More than 10 percent of the cells extracted from sandy sediments where water and nutrients can generally flow freely will proliferate when given a supply of nutrients in the laboratory. In contrast, less than one tenth of 1 percent of the cells drawn from sediments in the arid western U.S. (where the flux of water is minimal) will grow in a culture dish.

It may be that failure to culture most subsurface bacteria is a result of our inability to properly reproduce necessary conditions in the laboratory. Or perhaps these organisms are simply no longer alive. In rocks where the flux of nutrients and water is low, dead cells decompose exceedingly slowly, and so some of our biochemical assays would count them along with the few living cells. Alternatively, most of the organisms could be functioning but may have lost the ability to replicate.



JAMES K. FREDRICKSON

PIGMENTED BACTERIA inhabit parts of the subsurface near Idaho Falls, Idaho. Cultures of these microorganisms vary in appearance from purple to red because they produce copious amounts of a brightly colored substance that shifts in hue according to the ambient acidity.

The Prospects Underground

So far our colleague David L. Bulkwill of Florida State University has catalogued and preserved more than 9,000 strains of microorganisms from diverse subsurface environments. These isolates—containing a vast assortment of bacteria and about 100 types of fungi—are a source of novel microbial life that have not yet been fully tested for commercially applicable properties.

Of the small percentage of the collection that researchers have examined in detail, a surprisingly high proportion show potentially valuable capabilities. Examples of such traits include the ability to degrade toxic organic compounds as well as to produce antibiotics, heat-stable enzymes and even novel pigments. Pfizer is now screening 3,200 kinds of subsurface bacteria for the production of new antimicrobial products, and ZymoGenetics, a biotechnology company, is currently examining at least 800 isolates from this archive for production of other useful substances.

Perhaps many commercial products will result from these investigations. But even without such quick practical returns, the effort to probe the earth's interior for microorganisms will surely reward scientists with a fuller understanding of how life can exist in isolation from the surface. More study of subsurface communities may, for instance, indicate how life functioned on the early earth, before photosynthesis evolved. It may also provide insight into whether microbes might be living even now under the surface of Mars or below the icy exterior of some of the larger moons of the outer solar system. Seeing how microbes survive the rigors of deep burial on the earth, we are more inclined to believe tiny extraterrestrials might indeed be lurking out there. SA

The Authors

JAMES K. FREDRICKSON and TULLIS C. ONSTOTT conduct research for the Department of Energy's Subsurface Science Program. Fredrickson is an environmental microbiologist at Battelle, Pacific Northwest National Laboratory and also serves as editor in chief of the journal *Microbial Ecology*. He has specialized in applying molecular and isotopic methods to investigations of subsurface bacteria (including some obtained from his wine cellar). Onstott is a professor in the department of geological and geophysical sciences at Princeton University. His expertise is in studying the history of fluid and heat flow within the earth's crust. He began working with members of the Subsurface Science Program in 1993 to help determine the age of deeply buried microbial communities, and he quickly caught the mysterious subsurface bug that has infected this large group of scientists with a peculiar enthusiasm for their joint research.

Further Reading

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Information on the U.S. Department of Energy Subsurface Science Program is available on the World Wide Web at http://www.er.doe.gov/production/oher/EPR/subprog.html

