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Geobiologists: As Diverse as the Bugs They Study

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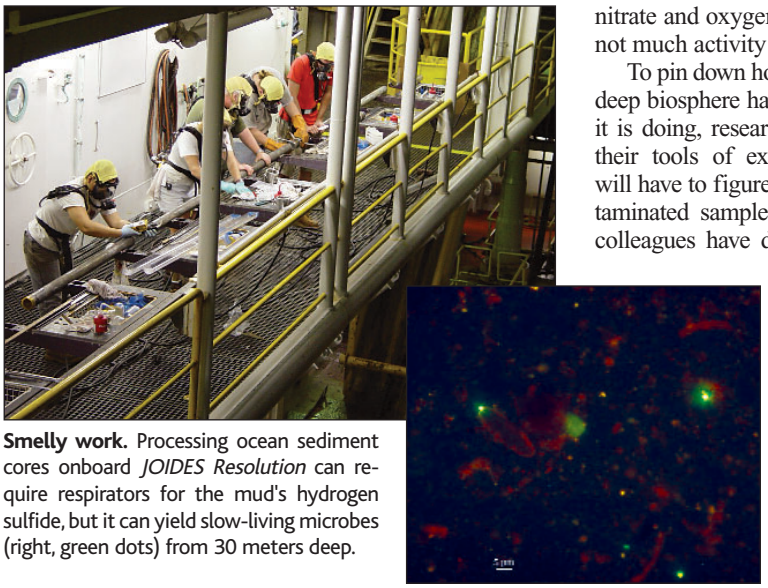
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the continental crust or the overlying sediments. Microorganisms are down there, but "we don't know what they're doing or what they're using for an energy source," says geochemist Jeffrey Alt of the University of Michigan, Ann Arbor.

When deep-diving oceanographers stumbled on a riot of life at sea-floor hot springs dotting the ridge crests in 1979, microbes were obviously a crucial part of it. More apparent evidence of vibrant deep microbial activity came in 1991, when oceanographers observed clouds of "fluffy white stuff" billowing from the East Pacific Rise following a volcanic eruption. After researchers found more flocs laden with thermophilic microorganisms pouring from recently disturbed hot springs, they concluded that "there's a biosphere below the sea floor," says oceanographer John Delaney of the University of Washington, Seattle.



Smelly work. Processing ocean sediment cores onboard *JOIDES Resolution* can require respirators for the mud's hydrogen sulfide, but it can yield slow-living microbes (right, green dots) from 30 meters deep.

Just how massive this ocean crustal biosphere might be remains unclear. Microbes are obviously active along the crest of the midocean ridge system, which stretches for 60,000 kilometers through the global ocean. For example, something seems to be nibbling on the glass that makes up about 5% of ocean crustal rock; samples of the glass brought up by deep drilling are scarred with pits filled with DNA-containing material (*Science*, 2 May 1997, p. 703). But the "snowblower events" of white floc billowing from ridge crests may not reflect a deep aquifer system "humming with life," as one oceanographer put it. On closer inspection, microbiologist Craig Taylor of Woods Hole Oceanographic Institution in Massachusetts found that the white stuff is not so much bacteria as sulfur filaments produced by bacteria consuming the hydrogen sulfide in hot spring waters. And the mats are probably the product of a brief "bloom" of bacteria feeding on a surge of hydrogen sulfide released by a quake or eruption rather than the release of a huge bacterial mass that's always feeding beneath the surface.

Microbes are making some sort of living at the ridge crest, where seawater heated to hundreds of degrees by underlying magma extracts the most chemicals from the fractured rock. But the crust cools as it spreads away from the ridge crest, and as the temperature drops, deep microbial life diminishes too. Ninety kilometers east of the Juan de Fuca Ridge, water rises through an ODP drill hole that penetrates 3.5-million-year-old crust. The water is 60°C, and microbiologists James Cowen of the University of Hawaii, Manoa, and Giovannoni of OSU find a concentration of cells less than that in seawater. A common misconception holds that "the deep-sea subsurface is packed with cells," says Giovannoni. "It's not; it's a fairly low biomass."

In even older, colder crust, life seems to have quit, or nearly so. The diffusion of oxygen and nitrate up from 40-million-year-old crust into the lowermost sediment, as found during the most recent ODP cruise, means that "whatever [biological] activity is going on in the crust isn't enough to strip out the nitrate and oxygen," says D'Hondt. "There's not much activity in old, cold crust."

To pin down how much life the bulk of the deep biosphere harbors and how much living it is doing, researchers will have to sharpen their tools of exploration. Oceanographers will have to figure out how to retrieve uncontaminated samples of ocean crustal life, as colleagues have done for marine sediments and continental crust. Then a means of gauging the pace of deep life must be developed. Culturing microorganisms in place will soon be attempted in deep mines, but molecular techniques for measuring gene expression may prove useful as well.

Deep-life researchers will also have to look for new sources of funding. Radioactive waste disposal in Sweden will keep work on deep granites going, and ocean drillers have made sedimentary and crustal life a focus of their next 10-year international drilling program (*Science*, 13 November 1998, p. 1251). But elsewhere attention is shifting toward shallow ground where microbes might help clean up pollutants. Life beneath the surface of Mars may get more attention than Earth's vast if thinly spread store of deep life.

–RICHARD A. KERR

NEWS

Geobiologists: As Diverse As the Bugs They Study

Derek Lovley and Kenneth Nealson have alternately sparred with each other and spurred each other on as leaders of the field they helped create

In the mid-1980s, Derek Lovley and Kenneth Nealson achieved something most scientists can only dream about: They put their stamp indelibly on a nascent scientific discipline. The two researchers independently announced the discovery of microbes that live off metals. The claims were surprising, even heretical, but the near-simultaneous findings "opened up a new field of study," particularly in the United States, says Yuri Gorby, a microbiologist at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. It turns out that these tiny metal-processing organisms have played a pivotal role in the vast sweep of geological history.

Since those early discoveries, Lovley's and Nealson's careers have followed similar trajectories. Today, the two men are considered intellectual leaders. Lovley chairs the microbiology department at the University of Massachusetts (UMass), Amherst; Nealson is now a distinguished geobiology professor at the University of Southern California (USC) in Los Angeles, and he works part-time in the astrobiology program at the Jet Propulsion Laboratory (JPL) in neighboring Pasadena. "There are a lot of parallels in what they have contributed and similarities in what they have worked on," says PNNL microbiologist Jim Fredrickson. Yet the two are opposites in personality and outlook, and according to numerous colleagues, their scientific and professional relationship is often characterized as one of intense rivalry.

Each has championed his own findings and the importance of the particular bacteria he first worked on 20 years ago. As a result, Lovley and Nealson have sometimes clashed at meetings and in print. "For a long time, the two weren't talking to each other," says

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William Ghiorse, a geomicrobiologist at Cornell University in Ithaca, New York. Even now, adds John Coates, a microbiologist at Southern Illinois University, Edwardsville, "there's no love lost between the two of them." Nealson and Lovley, however, don't like to talk about their conflicts, insisting that they are "really just differences in interpretation," as Lovley puts it.

Yet, as is often the case in science, they seem to have spurred each other on. Both have continued to make new discoveries, with Lovley in particular producing a blizzard of papers that have resulted in "phenomenal steps forward [scientifically] for the field," says Coates. Nealson has taken a more broad-based view, Coates adds, and "has been a tremendous spokesperson for geomicrobiology." Their individual successes—as well as progress in geobiology—speak to the fact that there's room for many temperaments in science. Nealson agrees: "Competition and healthy debate are the heart and soul of a field."

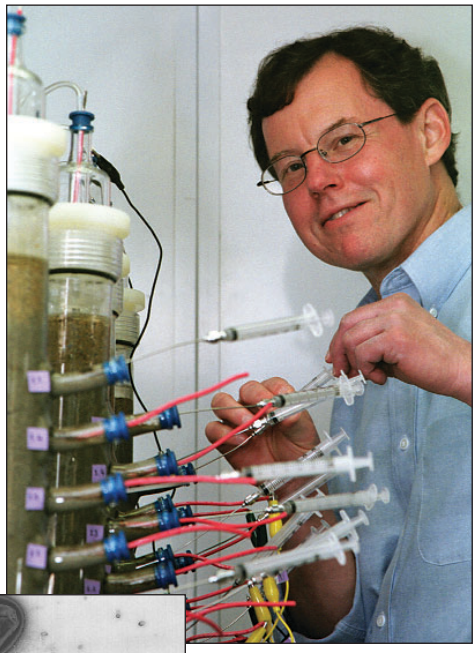
Lovley: Seeking the perfect microbe

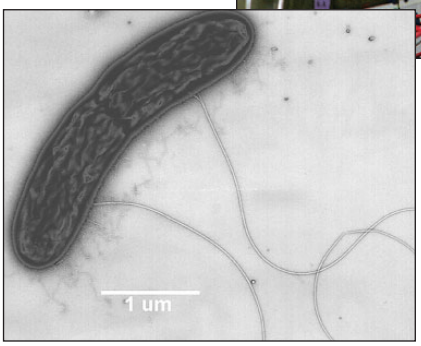
When Lovley joined the U.S. Geological Survey (USGS) in 1984 as a budding microbial physiologist, few had considered the possibility that microbes might "eat" or "breathe" metals. Lovley, however, was convinced such organisms exist. Earth's most abundant element, iron, provides a vast quantity of raw material that some microbes might thrive on. Lovley reasoned that in environments where no oxygen is present, microbes might convert insoluble ferric oxide to the more soluble ferrous form as they generated the energy they needed to survive. This would also mobilize iron, allowing the soluble form to move through the environment until it was eventually oxidized and made insoluble again.

Lovley set out sifting through river sediments, hoping to be the first to find bacteria that perform this feat. In just a few years he found what he was looking for: a class of bacteria he eventually called Geobacter. Since then, some 30 Geobacter species have been found in many types of sediments and soils, including deep in Earth's subsurface and in marine sediments. "We've been incredibly lucky," says Lovley.

Lucky—and hardworking, say his colleagues. Lovley churns out scientific discoveries like "a big rolling stone that you can't stop," says Cornell's Ghiorse. And he expects the same from his collaborators. Former Lovley postdoc Tim Magnuson, now a microbial physiologist at Idaho State University in Pocatello, remembers that when he worked with Lovley, the motto was "Finish an experiment a day and at least one publishable figure a week." "I thought it was a joke, but it was what he expected from his people," he recalls. That workaholic drive has paid off. Lovley has had a long string of publications in *Nature* and *Science*, and he continues to discover new organisms and metabolic pathways.

Lovley's studies of the biochemistry of Geobacter has borne out his early theories about their role in moving iron through the environment. In 1987, Lovley and his colleagues also found that some types of Geobacter help form a magnetic mineral called magnetite, which makes up a large percentage of rocks from 500 million years ago. These processes, says John Baross, a microbiologist at the University of Washington, Seattle, have "tremendous implications for virtually all of our mineral cycles on Earth."





A man and his microbe. Derek Lovley made Geobacter (*left*) a poster bacterium in geobiology.

Moreover, Lovley and his colleagues have shown that some Geobacter types use iron to metabolize complex organic compounds, explaining in part how the carbon in these compounds is recycled. Geobacter themselves "gain energy to grow from that process," says PNNL's Gorby, adding that this finding is Lovley's "seminal contribution." And in the early 1990s, Lovley and his colleagues helped prove that Geobacter is capable of processing gold or uranium.

To work their magic on iron or other metals, Lovley has found, Geobacter must first make direct contact with the metal. When iron is present in the environment, his team reported in the 18 April issue of *Nature*, Geobacter can grow flagella and home in on iron particles. Once they make contact, the microbes use a series of enzymes called cytochromes to move electrons from the interior to cell membranes for transfer to the iron, changing the metal's chemical state. The electron transfer helps generate adenosine triphosphate, which fuels all cellular activity.

The more Lovley learned about Geobacter, the more he began to think about harnessing it to clean up toxic wastes. He has found, for example, that Geobacter can degrade benzene and toluene, which might prove useful in bioremediation of oil-contaminated aquifers. He has also looked at contaminated soils and, in particular, at Geobacter's potential to alter the chemistry of uranium so that—in contrast to iron—it precipitates out of the water column. When Lovley moved from USGS to UMass in 1995, his research focus shifted somewhat. "When I started, I was more of a field person; I was not even sure I would buy an autoclave for sterilizing instruments or media for culturing organisms," he recalls. Now he runs an established but active lab focused in part on understanding Geobacter's lifestyle and biochemical makeup. He pushed to have the bug's genome sequenced, which he expects The Institute for Genomic Research (TIGR) in Rockville, Maryland, to complete very soon. As researchers merge field studies, lab results, and genome data, he says: "Things are coming together."

Nealson: Taking a broad view

While Lovley was making a name for himself with his studies of Geobacter, Nealson was concentrating on another versatile bug, a bacterium called *Shewanella* that also influences geological processes. He didn't start out with a geobiological bent, though.

As a young environmental microbiologist at Scripps Institution of Oceanography in La Jolla, California, in the 1970s, Nealson worked on luminescent bacteria that live in the light organs of fish. He discovered a protein called lux that was key to microbe-to-microbe communication, and he spent the next dozen years looking at how bacteria use lux to assess their density, a process called quorum sensing. He says his physical oceanography colleagues used to tease him, saying he should "work on

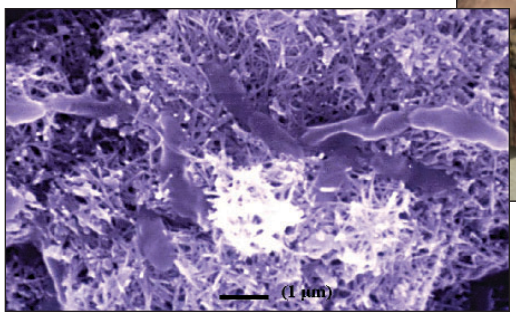
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something important"—such as Earth's geochemistry. In the early 1980s, he started to dabble in geobiology, specifically the geochemistry of manganese. He turned that into a virtually full-time endeavor in 1985 when he joined the Center for Great Lakes Studies at the University of Wisconsin, Milwaukee. He also imagined that lakes might be "more tractable" to study than oceans.

He was right. While tracking geochemical cycles involving manganese in Lake Oneida in upstate New York, Nealson says, "we lucked into an organism that lived by 'breathing' rocks," in particular iron and manganese oxides. The organism, part of a genus now named *Shewanella*, "breathes" manganese in the same way that Geobacter "breathes" iron. He and his colleague Charles Myers, now at the University of Wisconsin, Milwaukee, described this manganese-processing bug in 1988 in *Science*, just months after Lovley and his colleagues described microbial iron-reduction in *Nature*.

Nealson worked on *Shewanella* extensively for a decade, getting hooked on them in the same way that Lovley was hooked on Geobacter. As a result, Nealson's team "was instrumental in demonstrating the importance of these types of organisms in manganese biogeochemistry," says PNNL's Fredrickson: "He has great vision. He thinks outside the box very well."





From mud to Mars. In addition to showing that *Shewanella* was important in geobiology, Ken Nealson helped pioneer astrobiology.

To get an idea of *Shewanella*'s impact, Nealson measured manganese entering and leaving Lake Oneida over time. Based on the rate at which *Shewanella* processes manganese, he concluded that it played a big role in cycling this element through the environment. He also looked for *Shewanella* in different environments—and found new strains and species in many places, including the Black Sea. Nealson and his colleagues have since worked out how the 15 known species of *Shewanella* are related to each other and to other organisms.

Shewanella bacteria aren't hugely finicky, as it turns out. Nealson learned that they can interact with other minerals, for example, shifting electrons to iron even when that metal is locked in a clay called smectite. And when iron isn't available, the microbe can reduce sulfur and about a dozen other compounds, thereby helping recycle many different metals through the environment and changing the properties of soils. With Nealson's help, *Shewanella* became popular microbes for geobiologists to study in the lab.

Like Geobacter, *Shewanella* has become a leading model for bioremediation efforts. Nealson and others have shown that *Shewanella* can interact with chromium, uranium, cobalt, and nitrates, making it a possible candidate for cleaning up sites contaminated with several toxic wastes. To better understand these interactions, Nealson and his colleagues have been identifying specific proteins involved in transferring electrons from the cell to the different metals. In many ways, says Lovley, "we've studied the same issues in parallel."

After shifting from oceans to lakes, Nealson made another career change in 1998, turning to outer space. He moved to JPL to head up an interdisciplinary group of astrobiology researchers. Together, they developed the use of biosignatures—alterations made by organisms in rock texture, chemistry, and even appearance—as a means of detecting extraterrestrial life. "There's a lot of interest in methods we've been developing," he notes.

Nealson yearned to get back to his earthly, mineral-eating bugs in the lab, however. Although he still works part-time at JPL, he returned to USC as a researcher in 2001. He is now taking a more extensive look at some of the diverse *Shewanella* species both in the lab and in the field.

Champion microbes

Ever since Nealson and Lovley first published papers on their favorite organisms, people have asked the inevitable question: Which is more important—Geobacter or *Shewanella*? Lovley and Nealson have been staunch defenders of their particular bugs. On the one hand, Geobacter seems more common. "In every environment we look at, we see Geobacter; sometimes it's so abundant that it represents 50% of the microbes detected," says Lovley. In contrast, he says he rarely finds *Shewanella*. And his colleagues are impressed with all that Geobacter can do. The microbes will interact with "just about anything metal you give them," says Caroline Harwood, a microbiologist at the University of Iowa in Iowa City.

On the other hand, *Shewanella* "is a truly cosmopolitan organism," Nealson points out. It exists at very high pressures, for example. Furthermore, it can live in environments both with and without oxygen, and thus it can thrive right at the boundary between aerobic and anaerobic conditions. "That's where iron cycling is most active," says PNNL's Gorby. As a result, in some researchers' eyes, "*Shewanella* takes the prize for being flexible," says Cornell's Ghiorse.

However, "we don't know enough about the distribution of these organisms to say with confidence one is really more relevant than others," says PNNL's Fredrickson. Indeed, there may be other challengers waiting in the wings. For example, Joel Kostka, a microgeologist at Florida State University, Tallahassee, and his colleagues have recently isolated a class of Gram-positive bacteria from the same environments that are home to Nealson's and Lovley's favorites. "We really have very little information" about this class of organisms, says Kostka. "We've just scratched the surface of the diversity of organisms that are out there."

Lovley and Nealson, meanwhile, have moved far beyond the original studies that led them onto parallel paths in geomicrobiology. But in a few respects, the two are still competing. Each has embraced genomics to help elucidate how his favorite bug works, and each is racing to get his organism sequenced. TIGR started sequencing a *Shewanella* genome before it tackled Geobacter. But "[Nealson] was slow in the interpretation of the genome data," a prerequisite for publishing the completed sequence, says TIGR president Claire Fraser. When she hinted that Lovley's Geobacter genome might be published first, "that kicked Nealson into high gear," she says. Geobacter and *Shewanella* are in the final stretch, and everyone is expecting a close finish.

–ELIZABETH PENNISI

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