Pushing the Envelope of Life

A collection of unusual bacteria that thrive in conditions that would be fatal to all other organisms shows just how variable and adaptable life on Earth is

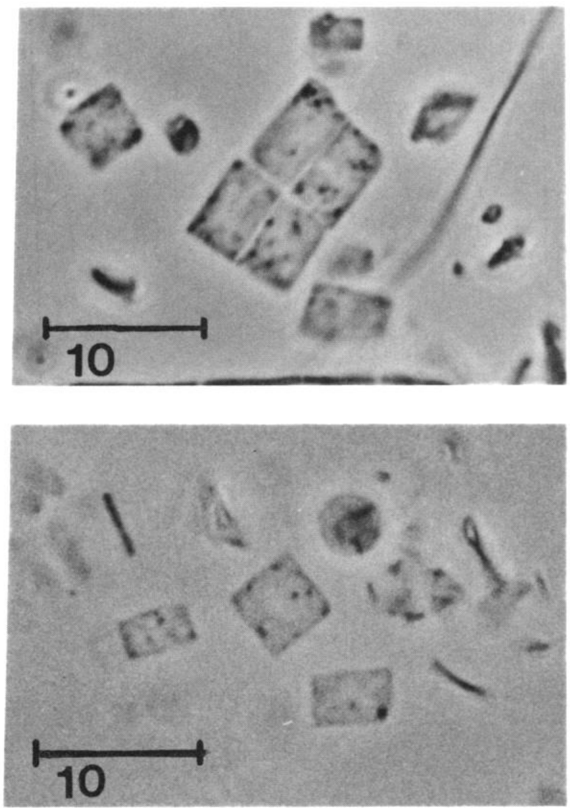
A SOLFATARA FIELD looks and smells and feels like something straight out of Dante's *Inferno*. Hot mud comes bubbling out of the ground, the air reeks of brimstone, and the few pools of water are polluted with sulfur and acid. It seems impossible that anything could survive here.

Yet something does. Here—and in equally unlikely places—live members of a group of microörganisms that seem almost too alien to have evolved on Earth. They are the archaebacteria—microbes that resemble normal bacteria but are actually no more closely related to those bacteria than they are to humans (see box on p. 159). Their most obvious characteristic appears to be a taste for extreme environments, which a large percentage of them inhabit. Various species live at temperatures above the normal boiling point of water, in lakes saltier than the Dead Sea, in water more alkaline than household ammonia or more acidic than gastric juices, and at the crushing pressures of the ocean depths.

Relatively little is known about the archaebacteria. Indeed, it has been only 13 years since they were recognized as belonging to an independent kingdom, separate from the eukaryotes, which include the higher forms of animal and plant life, and also from other bacteria, such as the familiar *Escherichia coli* or the pneumococcus bacterium that causes pneumonia. Scientists hope to learn from the archaebacteria how life adapts to extreme conditions and just what the limits to that adaptation are. These lessons may have some commercial value as well, for instance, by teaching the biotechnology industry how to design enzymes that work at higher temperatures and pressures than are now possible.

The most dramatic settings for archaebacteria are volcanic areas, both on land and sea. On land, the hot gases and sulfurous fumes that leak from Earth produce solfatara fields, which can be found in such places as Iceland, Italy, and Yellowstone Park. On the sea floor, hydrothermal vents spew out sulfurous gases along with water at temperatures that can reach hundreds of degrees centigrade.

It was at such an undersea vent off the coast of Italy that a group headed by Karl Stetter of the University of Regensburg, West Germany, discovered a group of archaebacteria that can grow at temperatures up to 110°C. This is the highest confirmed temperature at which any organism has been found to survive. But not only do members of the new genus, called *Methanopyrus*, survive at these temperatures; they demand them. The archaebacteria grow best at about 98°C, Stetter says, and they stop growing altogether if the temperature drops below 84°C.



Walther Stoeckenius

Postage stamp bacteria. *The plate-like archaebacteria like growing in brine ponds.*

Quite a few species prefer temperatures near or above 100°C, and nearly all are archaebacteria. The lone exceptions are the *Thermotoga* bacteria, which are a group of "normal" bacteria, or eubacteria, that branched off from the rest of the family very early; the *Thermotoga* can survive at up to 90°C. Many of these thermophilic microorganisms live on organic materials, combining carbon with hydrogen to form methane. Others get their energy by combining sulfur with hydrogen to form hydrogen sulfide.

Whatever their energy sources, each of these bacteria must have some way of preventing high temperatures from breaking down its constituent proteins and nucleic acids. Ordinarily, these large molecules come unraveled at temperatures above 60° or 70°C. But not in the archaebacteria, and this may have practical implications. Chemists, for instance, would like to discover how to make catalytic enzymes that operate at high temperatures, since higher temperatures increase the rate of chemical reactions.

The basis for the temperature resistance of the enzymes in archaebacteria is still unknown, however. They are composed of the same amino acids that make up enzymes in other organisms, Stetter says, "so it must be the overall structure that is different." But so far no one knows exactly what that difference is.

Archaebacteria must also have found some way to keep their DNA from unraveling. That may be prevented, Stetter suggests, by a type of histone-like protein discovered in the microbes. When the protein is added to DNA in vitro, the DNA can withstand temperatures 30° higher than usual. Another possibility is that the double strands in the organisms' DNA may be twisted more strongly than the DNA in other creatures, which would make it harder for the strands to separate.

The $64,000 question about these heat-loving archaebacteria is: How much heat can they stand? "There were people 20 years ago who would have told you things couldn't grow at 90°," says Norm Pace, a specialist in nucleic acid chemistry at Indiana University in Bloomington. Now researchers are competing to see who can find bacteria that beat the current record of 110°C.

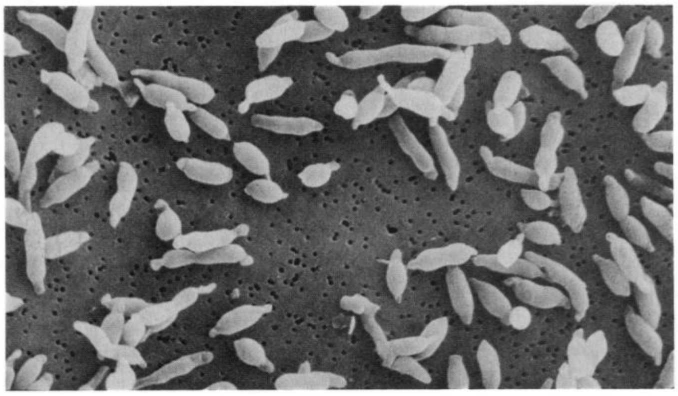
Pace says he has placed growth chambers over ocean hydrothermal vents and found evidence of colonies of bacteria growing at 130°C or above, but he must both confirm the water temperature and make sure that the objects he observed were actually the remnants of bacteria and not just artifacts. It wouldn't surprise him to find bacteria living at temperatures up to about 150°C, Pace says, but that is likely to be the upper limit on their heat tolerance.

If bacteria that prefer 100° water seem bizarre, consider the halophiles—or salt lovers—that thrive in water that makes the oceans seem fresh. Many bacteria can survive in solutions that are 36% salt, says Holger Jannasch, a microbiologist at Woods Hole Oceanographic Institution. At this percentage, the solution is saturated—no more salt can be dissolved into it. For the sake of comparison, the Atlantic and Pacific oceans are about 3% salt and the Great Salt Lake is about 25%. A high salt content is usually fatal to organisms because it draws water out of cells and disrupts the normal electrolytic balance.

Many halophiles have unusual shapes, says Walther Stoeckenius, a microbiologist at the University of California, San Francisco. Perhaps the most striking of these is a very thin, absolutely square archaebacterium. "When they grow," Stoeckenius says, "they elongate in one direction and then they divide so that they are square again." They remain next to each other after dividing and, because they elongate first in one direction and then in the other, "they end up looking like sheets of postage stamps" he says.

Salt-loving archaebacteria are most noticeable in evaporation ponds, where salt is harvested from sea water. A person flying over a cluster of these ponds is struck by their dramatic colors, from deep green to a striking red. In the early stages of evaporation, algae flourish in the ponds, giving them their green color, but as the salt content approaches saturation, the algae die off, leaving the halophiles, whose pigments provide the red color.

Some lakes in Egypt and other parts of Africa are not only extremely salty but are very alkaline, and archaebacteria have evolved to live in them as well. Stoeckenius says that some can survive in salt lakes with a pH of 11.5—nearly as alkaline as household ammonia.



Holger Jannasch

Heat lover. Thermatoga neapolitana *grows at temperatures up to 95°C.*

At the other end of the pH scale, some archaebacteria live in acidic solutions with a pH of 1 or less—more acidic than gastric juices but not quite as nasty as battery acid. Usually found near volcanoes or other areas where sulfur is abundant, these acidophiles oxidize sulfur to form sulfuric acid, and this waste product makes their environment extremely acidic.

Strangely enough, at least some of the acidophiles keep their interiors neutral, with a pH of approximately 7. No one knows exactly how they do this, Stetter says, but their membranes must be strong and have extremely good pumps.

Commercial mining companies often use acidophilic bacteria to leach valuable metals from low-grade ores. Gold particles, for instance, are often trapped in pyrite, an iron sulfide mineral otherwise known as "fool's gold," and the best way to extract them is to expose the ore to an acid solution containing bacteria that break down the pyrite. The gold can then be easily recoverd by precipitating it out of solution.

Many of the acidophiles also like high temperatures. The aptly named *Acidianus infernus* grows at temperatures up to 96°C and at pH levels as low as 1.

And then some archaebacteria are barophiles. They grow at the very high pressures found in the very deepest parts of the sea, as

The Third Kingdom of Life

Under a microscope, an archaebacterium looks just like any other type of bacterium. But looks can be deceptive. Genetic analysis has shown that the archaebacteria are distinctly different, enough so that they form another kingdom of life entirely.

Carl Woese at the University of Illinois in Urbana first recognized this in 1977. But, he says, "I made a mistake in naming them." That mistake has caused a great deal of confusion about the nature of the archaebacteria, he adds.

Before Woese's work, scientists recognized only two kingdoms: the eukaryotes, or those organisms, including plants and animals, whose cells have visible nuclei; and the prokaryotes, which included everything else—mainly bacteria and blue-green algae. Woese's analysis of many different bacterial species revealed that they could actually be divided into two kingdoms on the basis of similarities in their RNA sequences.

The members of one seemed to be the most primitive, because many of them were adapted to conditions that might have existed on a young Earth—high acidity, high temperature, high salinity, and a lack of oxygen. Woese called them "archaebacteria," or "primitive bacteria." The other group he labeled "eubacteria," or "good bacteria." Further work has shown, however, that calling both groups bacteria is misleading since—judging by the similarities and differences in the structures of their RNA—the archaebacteria are actually more closely related to the eukaryotes than they are to the eubacteria. Except for their affinity for extreme environments, however, the factors that make archaebacteria so different from the eubacteria are rather technical. They are very different at the molecular level, but their physical appearance is similar enough that no one realized they were not closely related until Woese did his RNA analysis.

This analysis also showed, Woese says, that the eubacteria, not the archaebacteria, are the more primitive kingdom. The eubacteria diverged from other lifeforms perhaps half a billion years before the archaebacteria and eukaryotes split up, he says.

Thus Woese blames himself for creating confusion about what archaebacteria are. And since he caused the problem, he feels it is only fair that he try to correct it, which he plans to do in an upcoming issue of the *Proceedings of the National Academy of Sciences*. He will propose a new set of names for the three kingdoms: bacteriotida, archaeotida, and eukaryotida. The archaebacteria will still be stuck with a "primitive" label—but at least they won't be called bacteria anymore. ■ **R.P.**

do other types of bacteria and even higher life forms, notes Aristides Yayanos of the Scripps Institution of Oceanography in La Jolla, California. In laboratory experiments, bacteria have been shown to grow at pressures of 1300 to 1400 atmospheres, which corresponds to ocean depths of 13 to 14 kilometers—deeper than the deepest point in the ocean, which is only about 11 kilometers. Some of these bacteria actually require such extreme pressure, Yayanos says. Certain barophilic species will not grow at pressures of less than 300 atmospheres, for instance.

And it's a good thing that the archaebacteria and other deep-sea organisms are able to resist such extreme pressures. Without their activities all the dead plant and animal matter that falls to the ocean floor would fail to decay. The activities of the barophilic bacteria thus help recycle organic matter in the ocean.

Nobody really knows what changes these organisms make in their molecular structures to adapt to such high pressures, Yayanos says, but scientists are slowly assembling clues. Yayanos and Ed Delong of Woods Hole have shown that deep-sea bacteria make less saturated membrane lipids as the pressure increases, an effect that could help maintain normal membrane fluidity in the face of the high pressures.

In addition, Stetter has found that certain archaebacteria modify the rate of production of several proteins at high pressure. The protein changes may reflect changes in gene expression. Recently, for example, a team including Yayanos cloned a gene from a deep-sea eubacteria that is regulated by pressure. The gene, which codes for a protein that seems to play a role in forming channels through the cell's membrane, is expressed at 280 atmospheres but not at 1 atmosphere. The researchers speculate that the bacterium modifies the membrane channels in response to increasing pressure, which would alter the diffusion of sugar nutrients and other molecules through the cell wall.

Perhaps the most important lesson that the archaebacteria offer is a more general one, however. They demonstrate just how robust life is. From the cold ocean depths to the heat of the solfatara fields—the closest thing to hell on Earth—life is everywhere.

■ ROBERT POOL

ADDITIONAL READING

D. Bartlett, M. Wright, A. A. Yayanos, M. Silverman, "Isolation of a gene regulated by hydrostatic pressure in a deep-sea bacterium," *Nature* **342**, 572 (1989).

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Fossils and British Pride

What is the difference between the most important paleontological discovery made in Britain during this century and a lump of coal? None whatever—at least according to Britain's Department of Trade and Industry. And that is why the oldest known fossil reptile may be on its way from Scotland to a German museum.

The specimen was found in a farmyard wall about 15 miles west of Edinburgh. The stones of the wall were quarried nearby in the 1830s, and there they remained, attracting little attention, until a professional fossil hunter named Stan Wood happened by in 1988. Wood noticed an intriguing fossil on the face of a 6 by 8 inch slab, and proceeded to buy the wall, thereby gaining title to the fossil, which he dubbed "Lizzie the Lizard."

Wood was being somewhat fanciful, because Lizzie is definitely not a lizard. More precise identification must await microdissection, but it is clear that the creature is a reptile. The fossil has been dated to 340 million years ago, which would put it at the beginning of the Carboniferous Era.

If that date is correct, it would push the origin of the reptiles back 40 million years, right into the so-called Age of Amphibians. Because the fossil is much better preserved than most early reptile specimens, it may give paleontologists a clearer picture of what the first members of that group were like.

On account of these considerable paleontological virtues, some folks in Britain feel that Lizzie ought to enjoy pride of place in a British museum. Alas, the price tag Wood has put on the fossil is just as considerable as its scientific significance: £180,000 ($290,000). At the moment it seems that no British museum can afford it. The Museum für Naturkunde in Stuttgart, with a full grant from the regional government, can.

In early December Britain's only hope seemed to lie with the Department of Trade and Industry (DTI). In some cases (such as those of Old Master paintings) the DTI has been persuaded to refuse an export license, keeping priceless bits of British heritage in the country.

On 8 December, the DTI heard expert witnesses describe Lizzie's importance and decided to delay granting an export license until April in order to give the National Museum of Scotland time to launch an appeal and come up with the needed cash.

Then, just before Christmas, the DTI's lawyers announced that the fossil didn't actually need an export license anyway. The existing legislation, they explained, covers only man-made artifacts, and although the fossil is very expensive, it isn't man made. If the legislation covered natural objects, one DTI man said, every export of British coal would have to be referred—and that would never do. (What the men from the DTI didn't explain is why the agency had held the hearing and ordered the delay in the first place.)

What happens now? Wood has an agreement with the museum in Stuttgart to deliver Lizzie, and he told *Science* he's sticking by that agreement "until I have an instruction from my client in Germany that they wish to do otherwise." Yet on the German side feelings are mixed. Rupert Wild, curator of vertebrate paleontology at Stuttgart, says, "I would rather that the specimen is kept in Britain because it is a British fossil."

The chances of Lizzie's remaining in the land of her birth (and death) now seem to rest on the National Museum of Scotland's raising the necessary wherewithal fairly quickly. Ian Rolfe, that museum's Keeper of Geology, says he is "still hopeful" about the prospects.

Lizzie herself is making a contribution to the cause. In March the Scottish museum is mounting an exhibition called "Dinosaurs Past and Present," which comes from the Los Angeles County Museum of Natural History. With permission from Stan Wood and from the museum in Stuttgart, Lizzie will be the star of the show—in the hope that she can earn enough to enable her to stay in Scotland.

Although the whole affair has raised some national hackles, British paleontologists don't seem too bothered by it, partly because the Stuttgart museum has promised that the fossil will be available for study. Indeed, some British academics, who would rather their names were not used, told *Science* that Lizzie might actually be better cared for in Germany.

■ JEREMY CHERFAS