SCIENCE NEWS 1999 June 12, 1999 Vol. 155 No. 24 p. 373

Prehistoric bacteria revived from buried salt

Hundreds of millions of years ago, before dinosaurs roamed the planet, an ocean rippled where the sun now bakes a New Mexico desert. As that seawater evaporated, its salt crystallized, sometimes trapping pockets of brine.

Covered by sediments over the years, those salt deposits currently sit half a mile below the desert floor. Microbiologists who have procured samples of the salt crystals and carefully tapped into the pockets, called inclusions, now claim to have isolated and revived bacteria that were last active 250 million years ago.

If true, the finding may shed light onto issues such as how life on earth evolved, where signs of life—past or current—on Mars might be found, and how long life-forms can survive.

"These organisms could very well be 250 million years old," says Raul J. Cano of California Polytechnic State University in San Luis Obispo. Four years ago, Cano's research group reported isolating living bacteria from the guts of dead insects entrapped in amber for up to 125 million years (SN: 5/20/95, p. 308).

That work, however, continues to draw skepticism from some scientists, who dismiss the idea that organisms can survive for eons. The new claims of ancient bacteria by William D. Rosenzweig and Russell H. Vreeland will likely provoke similar reactions. The two researchers, from West Chester (Penn.) University, head a group that for many years has been developing techniques to isolate bacteria from salt deposits without risking outside contamination.

Working in air-filtered laboratory facilities used previously for handling viruses such as HIV but not bacteria, the investigators sterilize the surfaces of their salt crystals with hydrochloric acid and sodium hydroxide. They then carefully drill into pockets within the crystals, withdraw the brine inside, and try to cultivate bacteria from the liquid. For the recent experiments, they procured crystals from the ancient salt deposits within the Waste Isolation Pilot Plant, a controversial underground holding facility for low-grade nuclear waste near Carlsbad, N.M. (SN: 11/1/97, p. 277).

At last week's meeting in Chicago of the American Society for Microbiology (ASM), the research team reported hitting pay dirt.

From brine in several different salt crystals, the scientists have grown novel spherical bacteria belonging to the genus of spore-forming microbes called *Bacillus.* Laboratory studies have shown that the bacteria can indeed form spores, which may explain their survival for hundreds of millions of years.

The researchers have identified an even odder microbe from the site, although they aren't as confident of its age. They cultivated the rod-shaped organism directly from dissolved salt crystals rather than from an inclusion.

The molecular contents of the second microbe's membrane suggest that it belongs among the archaea, the so-called third branch of life (SN: 8/24/96, p. 116). This reddish microorganism needs salt to maintain its cell membrane. Other salt-dependent archaea, called halobacteria, reside in such places as the Dead Sea and the Great Salt Lake.

Curiously, the newly isolated microbe derives energy only from molecules with a small number of carbon atoms, such as glycerol, acetate, and pyruvate. It has no use for more complex nutrients, such as glucose or amino acids. "It grows really well, but only on a limited diet," says Vreeland.

This simple metabolism, the researchers speculate, may reflect that of the ancient microorganisms first inhabiting the planet. The scientists have so far been unable to match the microbe's DNA to that of known creatures. "It's screaming how old it is," says Rosenzweig .

Yet proving the age of bacteria found in ancient salt deposits is no easy task. One major

concern centers on whether the fluid-filled inclusions formed when the original salt deposited or later, when heat, vibrations, or other events caused recrystallization.

"You have to be absolutely certain that the piece of salt . . . is 250 million years old and not just 100,000 years old or 20 years old," notes William D. Grant of the University of Leicester in England, who also examines ancient salt deposits for bacteria. He recommends that the researchers date the inclusions by analyzing the elements in the fluid they contain.

Vreeland counters that the New Mexico salt formation is one of the best-dated and most thoroughly studied and that his team worked with geologists to develop rigorous criteria for identifying original inclusions. From 220 kilograms of salt taken from the site, only 20 to 30 crystals, totaling less than 100 grams, met their requirements, he notes.

No matter how strict the precautions, some scientists dismiss all claims of ancient bacteria as modern contamination. "People are just going to laugh if you get *Bacillus* out of salt because *Bacillus* is everywhere," admits Grant.

Indeed, in the 1960s, a researcher named Heinz J. Dombrowski claimed to have revived *Bacillus* and other bacteria from salt deposits more than 500 million years old. Other scientists ridiculed him. "He was probably right, but he was ahead of his time," Grant now reflects.

Since then, microbiologists may have become more willing to accept claims of microbes lasting for eons since they have already identified organisms that thrive despite massive radiation, extremely cold and hot temperatures, and long periods without water. "A lot of paradigms about what life can and cannot do are coming apart now," notes Richard B. Hoover of NASA's Marshall Space Flight Center in Huntsville, Ala.

If the salt-derived microbes are hundreds of millions of years old, comparing their DNA with that of modern organisms should prove illuminating. "You can actually look at evolution on a molecular basis and do molecular paleontology. You're not dealing with just bones, but with genes," says Hoover.

NASA's interest in ancient salt deposits arises because Mars and Jupiter's moon Europa once had oceans and may have similar salt formations (SN: 11/1/97, p. 284). Missions searching for signs of extraterrestrial life might therefore target those deposits.

Meanwhile, the debate over ancient bacteria will persist. "There's going to continue to be healthy skepticism," says Melanie R. Mormile of the Pacific Northwest National Laboratory in Richland, Wash., who herself described 97,000-year-old salt-derived microbes at the ASM meeting, Whenever anyone claims they have revived organisms that are millions of years old, she says, "you've got to sit back and go, 'Wow, that's incredible. How can that be?' "

—J. Travis