In Pursuit of a Microscopic Methuselah
by Blake Edgar

Carrine Blank wants to find the common ancestor of everything. She may have already glimpsed her quarry, though its tracks are virtually invisible. In December, Blank, a microbiologist at the University of California at Berkeley, reported at the annual meeting of the American Geophysical Union that she had found a genetic fragment from a bacteria that appears to be closer to the root of the tree of life than any other organism.

The still unknown creature comes from Octopus Spring, not far from Old Faithful in Yellowstone National Park. For a few years now, Blank has been plucking primitive organisms from the park's near-boiling hot springs to study the temperature extremes at which life can exist. Her recent discovery marks the latest coup for molecular bioprospecting, which uses standard lab-bench techniques to locate new microbes in nature and elucidate their ecology. "Every time we go to a new environment," she says, "we find new things."

Using this approach, pioneered by Blank's thesis advisor, Norman Pace, it's possible to extract genes directly from natural environments and survey a site's microscopic biodiversity quickly and more completely than by cultivating bacteria in a lab one species at a time. Only a miniscule percentage of the world's microbes have yet been cultured in a laboratory. Prospecting for them in nature can reveal the broad pattern of what's out there, especially in Earth's most remote and seemingly inhospitable niches. Then biologists can pinpoint specific fragments of a gene that will either match with a known organism or turn out to be something completely new.

In Yellowstone hot springs lives the critter closest to the root of all life.

For most people, Yellowstone evokes images of bison and bears, but the park is also renowned for the most minute denizens of its many hot springs and geyser vents. Octopus Spring contains one of the richest microscopic mother lodes. In 1965, biologist Thomas Brock first demonstrated that the springs blossom with bacteria when he began collecting them on glass slides suspended in the water. One of the creatures Brock identified, Thermus aquaticus, contains an enzyme called Taq polymerase, which later became the basis for the polymerase chain reaction (PCR)--the lab technique that revolutionized molecular biology and now makes research like Carrine Blank's possible.

Thirty years after Brock made his first forays in Yellowstone, Blank arrived at clear, blue Octopus Spring. Using a test tube attached to one end of a long pole, she scooped samples of sediment from the bottom of its three-foot-deep source pool, where an underground vent releases hot gases and roiling water into the spring. Each sample was then frozen on site.

Back at her lab, Blank began the tedious task of teasing bits of DNA away from silica in the sediment.
The next step uses PCR to make millions of copies of a particular gene from RNA, another genetic molecule. If DNA is the foreman of protein production, then RNA corresponds to the assembly-line workers who actually make the stuff. Blank is interested in the gene that encodes RNA found inside ribosomes, the cellular factories where proteins get assembled.

Ribosomal RNA (rRNA) has proven to be a powerful and reliable molecule for building evolutionary trees of life. Such trees led a trio of biologists to propose in 1990 that all life could be divided into three broadest categories, or domains: the Archaea, the Bacteria, and the Eucarya, the last of which lumps plants, animals, fungi, and most any other critter that springs readily to mind, unless you're a microbiologist (see Horizons, Pacific Discovery, Fall 1994).

The take-home message is that the vast majority of life goes unseen by human eyes; while their external forms may vary little when compared with, say, a sequoia and a dandelion, the microbes comprising Archaea and Bacteria nonetheless contain most of the planet's genetic diversity. Yet only about 5,000 species in these domains have been described.

By late 1996, Blank had mapped some sequences of rRNA for genes extracted from Octopus Spring. She was ready to compare them to a computerized database of 6,000 microbial rRNA sequences from known organisms. For the sequence labelled OctSpA1-106, the computer came back with a startling identification: the sequence fell smack between the archaeal and bacterial branches on the tree of life, a spot where nothing had previously been known. "That told me immediately that this thing is different and is going to be interesting," says Blank. Even though earlier surveys of Yellowstone hot spring RNA by Pace and his colleagues had yielded the deepest (or oldest) diverging twigs in both the Bacteria and Archaea domains, Blank still initially didn't believe her results.

The sequence is distinct enough, though, Blank says, that it warrants being designated a new kingdom of life, a level of diversity akin to animals or plants. As to where Octopus Spring 106 falls within the even broader three domains of life, more than three-quarters of the sequence's nucleotide signatures—chemical clues used to distinguish each domain—ally 106 with the Bacteria, so it sits squarely on that branch. Evolutionary trees constructed from various molecules place the tree of life's root, the branching point for the ancestor of ancestors, among the Bacteria. This was the first domain to evolve, and the Archaea and Eucarya are closer relatives of each other than either is to Bacteria.

Six percent of the signatures in Octopus Spring 106 are unique, and 15 percent link this new sequence with the Archaea. The closest apparent relatives of 106 belong to a bacterial kingdom called Aquificales, whose members generally show much less similarity to Archaea. That 106 appears to be twice as similar to the Archaea as any other kind of Bacteria further suggests that this critter emerges from close to the root of all life.

Based on the chemistry of Octopus Spring and 106's apparent position in the grand swath of life, a very rough portrait of the microbe can be sketched. It is most likely hyperthermophilic, or heat-loving, and may rely on hydrogen gas for food and energy. In other words, it thrives in the sort of conditions that prevailed on Earth when life first arose. But Blank hopes to fill in some details. "We have a sequence and we have a lineage on a tree. It's pretty obscure," she admits. "That's one reason we want to paint a face on this creature."

She and collaborator Sherry Cady of NASA Ames Research Center in Mountain View observed three dominant microbial shapes in the one-cell-thick film from Octopus Spring. Blank plans to insert fluorescent probes tailored to her mysterious sequence inside the three kinds of microbes. The probe will bind to the RNA in each one, and if it finds an exact match, that microbe will start to glow under a

fluorescence microscope.

Blank suspects that the 106 sequence belongs to a creature made up of very thin filaments between 30 and 60 microns long. At one end the microbe secretes an adhesive holdfast able to withstand boiling water. Who knows? Maybe the next super glue or some other useful product will come of Blank's prospecting efforts. Until then, it suffices to realize that somewhere out there, perhaps within the border of a national park, the close kin of Earth's earliest life continues to endure.

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