Discovering a new life form in the hot springs of Yellowstone

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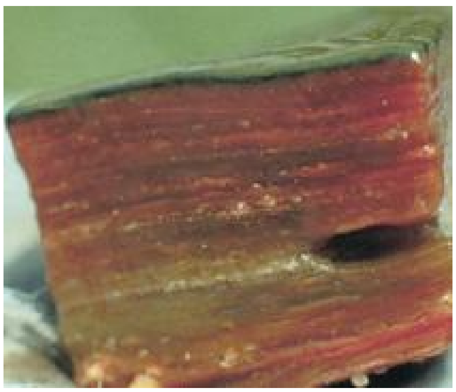

Cross-section of a microbial mat. Image: David M. Ward

Geysers, mud pots, steam vents and hot springs in the region now known as Yellowstone National Park awed American Indians and early European explorers. Now, two million tourists visit the park in northwestern Wyoming each year to watch wildlife and view the spectacular scenery. Scientists home in on the hot springs, exploring their ecology and plumbing their scalding waters in search of highly adapted, heretofore-undiscovered microorganisms.

"Octopus Spring and Mushroom Spring in Yellowstone are two of the most thoroughly studied hot springs on the planet," said Don Bryant, Ernest C. Pollard professor of biotechnology at Penn State. Yet in these two unwelcoming habitats Bryant and David Ward, a microbial ecologist at Montana State University, found a new thermophilic, or heat-loving, bacterium that survives by transforming light into chemical energy. Bryant, who has studied bacterial photosynthesis in an academic career spanning more than three decades, characterizes finding this new chlorophyll-producing microbe as "the discovery of a lifetime."

Bryant and Ward described the new bacterium, Chloracidobacterium thermophilum (Cab. thermophilum, for short) in a paper published in the July 27, 2007, issue of Science. Not only has the microbe been declared a new genus and species, it is the first phototrophic member of the phylum Acidobacteria. Phyla are major taxonomic groupings of organisms, and there are about 50 phyla of bacteria. Before Bryant and Ward discovered Cab. thermophilum, only five of those phyla included chlorophyll-producing members. Now, Cab. thermophilum has placed the acidobacteria among the ranks of the chlorophyll producers. (In terms of biomass, chlorophyll-producing bacteria are tremendously abundant, and they are crucial to our planet's ability to sustain life, performing an estimated half of all photosynthesis on Earth.)

Some like it hot

Yellowstone's hot springs show vivid yellows, oranges, reds, ochres, greens and blue-greens. Many of these colors are pigments produced by the different species of bacteria that exist on and within the roughly inch-thick microbial mats that form in and near the springs' runoff channels. Octopus Spring earned its name from its complex shape. It's an alkaline spring, with a pH of 8.5 (the pH scale ranges from 1 to 14, with 7 being neutral: neither acidic nor alkaline). Octopus Spring also has a high content of dissolved silica and a relatively low sulfide content. Its geothermally heated source releases 95-degree C water (203 degrees F) — very near boiling at the high altitude of Yellowstone, in the Rocky Mountains.

Octopus Spring has two effluent channels in which the water, flowing away from its source, gradually becomes cooler: 66 to 50 degrees C, or 151 to 122 degrees F. In those channels float the gaudy mats that Bryant and Ward have been studying, in areas where the effluent cools to below 72 degrees C, the highest temperature at which photosynthesis can occur.

The only living inhabitants of Octopus Spring are its microbes; the place is simply too hot for higher organisms to survive. A microbial mat is a world of its own, consisting of many layers and interlacing zones that support a range of different microorganisms. "The mat looks a bit like lasagna when you cut into it," Bryant says. “In the one that we’ve been working on, you can move one centimeter away from our study area and find a bacterium that looks physically the same as a neighboring type, yet is sufficiently different genetically to be considered a separate species.

"We’ve been looking mainly at the upper two millimeters — the green part of the mat and what’s just beneath it." Cab. thermophilum grows in this surface area, along with cyanobacteria. (Cyanobacteria are an exceedingly common and abundant group of bacteria also known as blue-green algae. They exist in the oceans, where they play a key role in the marine nitrogen cycle, and in a wide range of freshwater habitats. Scientists believe their oxygen-releasing photosynthesizing made it possible for life to evolve on Earth.)

In Octopus Spring, "the upper layer of the mat, exposed to light and the atmosphere, is where all of the photosynthesis takes place," Bryant said. "Other microorganisms in the lower layers of the mat live under anoxic conditions and do not carry out photosynthesis.

"The mats are complex communities," continued Bryant, "with many synergistic relationships between the various microbes." Because of those complicated and often poorly understood relationships — as well as the hard-to-duplicate physical and chemical characteristics of the hot springs habitat — thermophilic bacteria can be difficult or even impossible to grow in the lab. "Cab. thermophilum simply refuses to grow on an agar plate," Bryant said.

In the hot springs, Bryant and Ward and their graduate students and colleagues take samples from the mats using low-tech methods: A spatula works nicely for cutting out a portion of the mat, as does a coring tool. (Neither device causes lasting damage, as the microbes grow back quickly.) Once a sample is brought into the lab, the target layer is removed. The researchers then use a technique called metagenomics to explore the organisms living in that part of the mat, and to puzzle out their physiology, metabolism and ecological relationships.

How little we know

In traditional microbiology and microbial genome sequencing, researchers isolate bacteria by growing them as pure cultures on media such as agar — the classic culture in a test tube or a petri dish — and then take DNA from the pure strain and sequence it. Metagenomics is based on a completely different approach: DNA is harvested from the vast numbers of bacteria in an environmental sample and then analyzed through DNA sequencing, almost as if it were part of the genome of a single organism. The technique, developed in the 1990s, involves extracting DNA and then subjecting it to "shotgun sequencing," in which many relatively short DNA sequences are assembled into "consensus sequence fragments," or "contigs," through the use of powerful computational techniques. Said Bryant, "The sequence contigs can then be further analyzed to reveal information about the organisms living in that environment, and also to infer those organisms' physiological and metabolic processes."

In sorting through their Octopus Spring samples, Bryant and Ward focused on two genes: 16S ribosomal RNA, part of the machinery used by all living cells to manufacture proteins, and distinctive from species to species; and the gene coding for a protein called PscA, which, because it is essential for converting light energy into chemical energy, is an indicator of photosynthesis.

"Finding two distinctive genes among thousands of DNA fragments is not enough to justify naming a new organism," Bryant said. "You need to prove that those genes came from the same genome, from the same organism" — this in a part of the mat where no less than six phototrophic bacteria have already been identified. It turns out that the genes for PscA and 16S ribosomal RNA are physically close together in the chromosomes of Cab. thermophilum, and the research team was lucky enough to isolate a single large DNA fragment that included both. The odds of making such a connection were not high.

In addition to finding Cab. thermophilum in Octopus Spring, the microbiologists also retrieved it from nearby Mushroom Spring and from Green Finger Pool in the Lower Geyser Basin not far from the famous Old Faithful Geyser. Ronald Weiner of the National Science Foundation (NSF) characterized the discovery of Cab. thermophilum as "an excellent example of how metagenomic information reveals how little we know about life on Earth." The NSF had helped to fund Bryant's and Ward's Yellowstone project, along with the U.S. Department of Energy, NASA's Exobiology Program, and the Thermal Biology Institute of Montana State University.

Before Bryant and Ward fished Cab. thermophilum out of Octopus Spring, most acidobacteria had been found in poor or polluted soils that were also highly acidic, often with a pH below 3 — hence the phylum's name. But Cab. thermophilum inhabits an alkaline, or basic, environment. Said Bryant, "When we look at the 16S ribosomal RNA sequences of other thermophilic bacteria, it appears that close relatives of Cab. thermophilum also exist in Tibet and Thailand," in hot springs that are chemically similar to those in Yellowstone. In fact, Bryant speculates, additional relatives may turn up in microbial mats of hot springs worldwide.

The light gatherers

The greens and blues in Octopus Spring's microbial mats come from chlorophyll. Cab. thermophilum carries its chlorophyll in antenna-like structures known as chlorosomes, each of which holds an estimated 250,000 chlorophyll molecules.

"Think of a chlorosome as a sac of chlorophylls," said Bryant. "In Cab. thermophilum, the chlorophylls don't use a protein scaffold, as in other photosynthesizing organisms. This bacterium has evolved a unique way of presenting its chlorophyll to the sun, and it does so on a scale vastly larger than other photosynthetic organisms. This means Cab. thermophilum can photosynthesize in lower-light situations — we estimate about a thousand times lower than the light required by a blade of grass." He added, "Cab. thermophilum is a distinctly different phototroph. The proteins that actually do the solar energy conversion process are quite different from any previously described."

Cab. thermophilum makes three types of chlorophyll, something that allows it to live productively and in close proximity with its microbial neighbors. "By having chemically distinctive chlorophylls," said Bryant, "Cab. thermophilum can absorb different light wavelengths, so it can thrive in the same place in the mats as the cyanobacteria, and even underneath the cyanobacteria. These different microorganisms do not compete directly with each other for light," a situation that allows the community as a whole to be more productive.

The orange, reddish, and brownish colors in the microbial mat come from carotenoids. These molecules function as auxiliary light-harvesting pigments, and they also provide protection from the intense sunlight in this high-altitude setting, and from toxic oxygen compounds. "In addition to having plenty of green chlorophyll, Cab. thermophilum is a major carotenoid producer," Bryant said.

"During the day, when sunlight reaches the mats, the cyanobacteria start producing oxygen — so much of it that the mat actually begins to give off bubbles of oxygen in the first few hours after sun-up. When photosynthesis is in high gear, the environment in the mat has about five times the oxygen present in the atmosphere. “This supersaturation with oxygen requires that these microbes protect themselves," said Bryant. "This is a really toxic situation — you might call it too much of a good thing."

Bryant's colleague David Ward has been studying the ecology of the microbial mats at Octopus Spring for more than 30 years, since he was a graduate student under Tom Brock of the University of Wisconsin. (It was Brock who discovered Yellowstone’s most famous microbe, Thermus aquaticus, from which the heat-stable enzyme known as Taq polymerase was later isolated. Taq polymerase has revolutionized science by making the polymerase chain reaction, or PCR, a routine procedure, widely used today for sequencing and analyzing genes, diagnosing hereditary diseases, identifying genetic fingerprints in forensics and paternity testing, and many other applications — including finding new photosynthetic microbes.) As a professor of Microbial Studies in the Thermal Biology Institute and the Department of Land Resources and Environmental Sciences at Montana State, Ward works to understand the functioning of the mats and the interrelationships between the resident microbes.

"During the day, at least seven different types of organisms in the mat conduct photosynthesis," Bryant said. "They convert carbon and oxygen into sugar polymers, such as glycogen — effectively carbo-loading. Then at night, they feed on the stored carbohydrates made during the day." Some photosynthesizing bacteria switch to fermentation after oxygen has been consumed, producing organic acids, foods that are released into the mat to be used by other community members. Cab. thermophilum probably relies on such products supplied by the cyanobacteria, which ferment at night when they're not photosynthesizing. Returning the favor, Cab. thermophilum removes these organic acids from the mat environment that could accumulate and inhibit the growth of the cyanobacteria, which don't do well under acidic conditions.

Seeing things fresh

Said Bryant, "We have no clue whether Cab. thermophilum will prove useful in medicine or biotechnology or for cleaning up pollution," as have other heat-adapted microorganisms discovered in recent years. "But every time science discovers another organism that converts sunlight into energy, we improve our knowledge of the evolution and the inner workings of photosynthesis."

Today, researchers are looking at microbes as a potential source for producing energy on a large scale. "A better understanding of how different organisms photosynthesize, and how they work together in nature, may help us figure out how to better capture and convert solar energy," said Bryant. "To optimize biomass generation, you have to find out the community dynamics — who's there and how they all work together."

During summers to come, Bryant and Ward will continue their studies in other hot springs in the Yellowstone Basin, an area considered to be one of the most diverse geothermal sites in the world. "We hope to find and identify other bacteria that haven't been described yet," said Bryant.

Recently, he and Ward have been working to more closely characterize Cab. thermophilum. "From its genome sequence," said Bryant, "we've identified a gene that completes an important biosynthetic pathway for the synthesis of bacteriochlorophyll c," a type of chlorophyll pigment. “We also hope to develop a molecular-level understanding of Cab. thermophilum’s antennae, the organism’s light-harvesting apparatus." From samples sent by Bryant and Ward, colleagues at the University of Groningen in the Netherlands have produced images of structures that look oddly like spinach rotini — a unique way of presenting chlorophyll to sunlight in order to capture photons.

Bryant reflects on the close friendship that has grown between him and his fellow researcher Ward. "This discovery of a new life form was made because I broke out of the mold of what I usually do in the lab," he said. "In Yellowstone, I interacted with someone who has a different view, a different scientific perception of the natural world than I have.

"I'm much more of a biochemist and a physiologist, while Dave is a microbial ecologist. Aside from both being microbiologists, we don’t do the same things at all — we don’t even speak the same scientific language, in many cases. But by embracing modern DNA sequencing techniques, we’ve found a common ground. This is truly synergistic research."

Provided by Research Penn State

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