

Uranium-contaminated site yields wealth of information on underground microbe community

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University of California, Berkeley, scientists have sequenced nearly all the genes in an underground community of microbes at a contaminated uranium mill site in Rifle, Colo., providing information that could help scientists better manipulate the microbes that remediate heavy metal contamination or those that take up and store carbon from the atmosphere.

Each of the 150,000 genes from the sample was assigned to one of 80 different microbes in the soil, an unprecedented computational feat made possible by new [genomic tools](#) developed at UC Berkeley.

The findings could help improve clean-up at hundreds of sites around the United States where microbes are nurtured to convert [toxic metals](#), including arsenic and mercury, into chemical forms that will not leech into aquifers and streams, the scientists said. It may be possible, for example, to add nutrients that would create the ideal mix of microbes to immobilize the metals, instead of just feeding all the microbes already present.

"In order to make this goal a reality, it is critical not to just detect the relevant genes in subsurface microorganisms, but to know enough about the lifestyles of the organisms with genes of interest so that manipulation of the system enriches specifically for that organism," said study leader Jill Banfield, UC Berkeley professor of earth and [planetary science](#) and

of environmental science, policy and management.

The information in the genomes of the 80 microbes located 10 feet or more beneath the surface could also lead to improved methods for stimulating the uptake of carbon from the atmosphere by [soil bacteria](#) to reduce [greenhouse gases](#).

"Our study turned upside down what we thought was happening at the bioremediation site," said lead author Kelly C. Wrighton, a UC Berkeley post-doctoral fellow who specializes in the physiology of microbes.

"What these genomes have given us is amazing in terms of being able to look under the hood at the machine of these organisms that we never really knew anything about, except that we saw them in certain types of environments."

Wrighton, Banfield and colleagues at UC Berkeley, Lawrence Berkeley National Laboratory (LBNL), Oak Ridge National Laboratory (ORNL) and Pacific Northwest National Laboratory (PNNL), report their metagenomic analysis in the Sept. 28 issue of the journal *Science*. Banfield is also a member of the Earth Sciences Division at LBNL.

The metagenomic analysis proves that methods developed by Banfield at UC Berkeley to take a jumble of sequenced genes and assign them to specific microbes works even when there are nearly 100 bacteria and Archaea in the sample. Most metagenome analyses generally leave the genes unassigned to organisms, which gives scientists little idea of the role specific microbes play.

"The techniques developed in Jill's lab for doing metagenomics of very complex microbial communities is giving us a much more accurate look at what the microbes are doing and how we can modify them to get them to do more effectively what we want them to do, which is reduce uranium to form a solid," said Philip E. Long, an LBNL geologist who

manages research at the Rifle study site. "We are finding out from these studies that the subsurface microbial economy is different from what we thought."

The "dark matter" of biology

The microbes came from groundwater samples taken at a site once used to process vanadium and, during and after World War II, uranium. The site borders the Colorado River, which means that rain can carry dissolved metals into the groundwater and eventually into the river. Some microbes "breathe" the metals like we breathe oxygen, chemically altering them so they become insoluble and remain in the sediment, Wrighton said.

Banfield refers to microbe communities like these as the "dark matter" of biology, an analogy to the missing mass in the universe that has stumped astronomers for decades. The bacterial tree of life can be divided into about 60 phylum-level branches, but essentially nothing is known about half of them, she said.

"This new study provides new knowledge about the ecology as well as the evolution of a significant chunk of what could be considered the dark matter of the microbial world," she said.

Before now, Banfield had performed metagenomic analyses of eight microbes coexisting in highly acidic underground streams in a former California mine and current Superfund clean-up site. Such an analysis involves grinding up all organisms in a sample, sequencing all the genes and then matching each with a unique microbial species.

The new study involved the genomes of 10 times more organisms. All are anaerobic – they don't breathe oxygen like most organisms on Earth – and most, while not new to science, are totally unstudied because they

cannot be cultured in the laboratory. Many are only a few hundred nanometers across, making them among the smallest known microbes.

Scientists at the Rifle site spread acetate – essentially dilute vinegar – in the subsurface to feed the underground bacteria that convert soluble metals to insoluble metals. They had assumed that they were culturing a colony comprised mostly of *Geobacter bemidjensis*, a well-known metal-reducing bacterium.

Instead, Wrighton said, analysis of three samples obtained within 10 days of acetate application showed a healthy population of *Geobacter*, but a throng of other bacteria presumably feeding on dead *Geobacter* and other carbon in the soil from previous additions of acetate. These organisms use or ferment complex carbon, such as dead plants and dead microbes, and produce hydrogen, small organic carbon compounds and carbon dioxide.

"The fermenters producing hydrogen after multiple additions of acetate may be more important to the underlying microbial community than we once thought," Long said.

"What we found was like a microbial zoo," Wrighton said. "We thought that the respiring organisms – those breathing metals and making carbon dioxide – were our heavy lifters, but we found that fermentative organisms probably underpin their metabolism. These organisms produce as a bi-product acetate, lactate and ethanol as well as hydrogen and those are all components that these respiring organisms can use."

Wrighton, Banfield and their team are continuing metagenomic analyses of samples from the Rifle site, including some obtained before nutrients are added to determine what the natural population looks like. This will provide a comprehensive view of the metabolic potential of the subsurface that ultimately can be harnessed for bioremediation, Banfield

said.

"Our research has lifted a veil on a large portion of bacterial life and enabled us to probe in great depth and detail these unknown and uncultured bacteria," said Wrighton, who hopes to use this information to raise the [microbes](#) in the laboratory. "We now have information, which is encoded in their genomic DNA, pertaining to what they look like, how they make their living in the environment, and the interactions they have with other organisms."

More information: "Fermentation, Hydrogen, and Sulfur Metabolism in Multiple Uncultivated Bacterial Phyla," by K.C. Wrighton et al, *Science*, 2012.

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