

How microbial communities thrive in hydraulically fractured shale wells

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Samples of the fluids produced at a hydraulic fracturing site. Credit: Kaela Amundson, CSU

In survival game shows, contestants are whisked away to a foreign location, where they face unfamiliar stresses. To stay in the game, they must adapt to the surroundings and often need to work together with fellow competitors.

As it turns out, the same is true on the microscopic level for microbes, such as bacteria and viruses, residing in fluids from [hydraulic fracturing](#) or fracking processes. The conditions in these deep underground environments are so constrained scientists didn't think life could survive there, let alone thrive.

But a few years ago, a small team of researchers began looking closely at

these engineered worlds. The team's findings are not only surprising, but also informative to the fracking industry. Such worlds could even be considered as a model system for understanding how microbial community members interact within their ecosystems.

Some of the research team's work is part of a 2015 Facilities Integrating Collaborations for User Science, or FICUS, project called "Microbial Controls on Biogeochemical Cycling in Deep Subsurface Shale Carbon Reservoirs" led by researchers then at The Ohio State University.

The FICUS initiative resulted from a collaborative effort between two Department of Energy Office of Science user facilities: the Environmental Molecular Science Laboratory, or EMSL, located at Pacific Northwest National Laboratory (PNNL) in Richland, Wash., and the Joint Genome Institute, or JGI, in Walnut Creek, Calif., managed by Lawrence Berkeley National Laboratory.

The multidisciplinary team also includes researchers from Colorado State University (CSU), the University of New Hampshire, West Virginia University, and The University of Maine. Since the initial proposal, the researchers have shifted their direction as the science takes them along, publishing a handful of high-profile papers.

"It's a novel system," says team member David Hoyt, a biochemist at EMSL. "And the nice thing about this particular set-up is that for the microbes that do survive this harsh environment, we get a lot of testable relationships that can tell us what types of molecular strategies the organisms have to use."

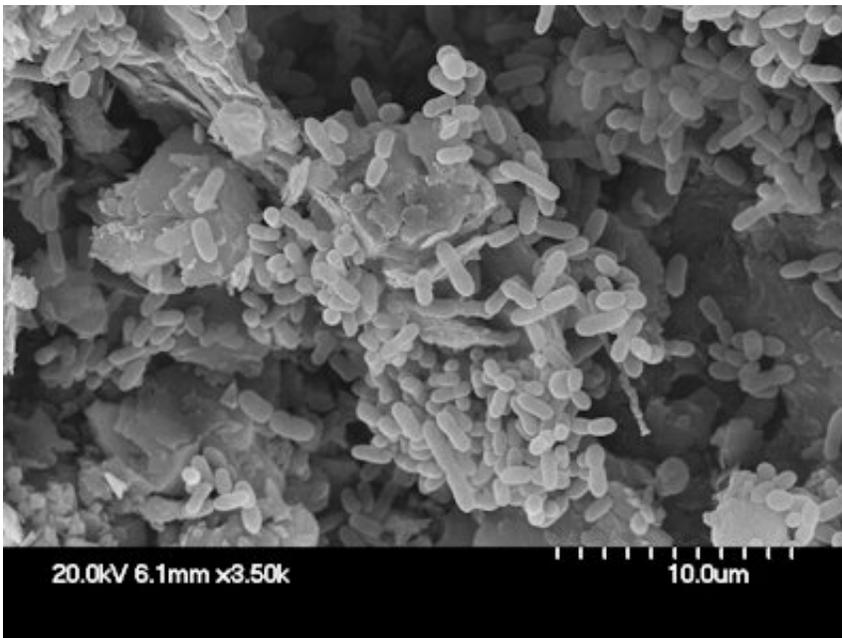
Surprises in the extreme

Fracking is a decades-old industrial technique applied to deep wells to unlock natural gas and petroleum from rock formations, such as shale,

for extraction. Recent years have seen a rise in using the technique due to improvements in horizontal drilling.

The one-time disturbance uses high-pressure fluids to create hairline cracks, or fractures, in the subsurface rocks. The fluid is a mixture primarily of water with chemical additives and sand materials to keep the fractures open, so the desired components can continue to flow out.

The research team looked at "pristine" shales – those not subjected to fracking – and found no detectable evidence of active microbial life, according to team member Michael Wilkins, a former PNNL scientist, now a geomicrobiology professor at CSU. "These shales are basically sterile," says Wilkins, "and suddenly during the hydraulic fracturing process, microorganisms are introduced into the shale – by accident."



Bacteria cells growing on shale particles. Credit: Michael Wilkins, CSU

At this point, often more than a mile and a half underground, the conditions are harsh. The temperatures are around 65 degrees Celsius, and the salt levels are four times that of normal seawater. The pressures are roughly 500 atmospheres – 15 times the amount of pressure felt by a human in the deepest recorded SCUBA dive.

And yet, some of the microbial "contaminants" manage to survive and, as the researchers learned through time-series experiments, colonize their ecosystems.

Team member Kelly Wrighton, a soils microbiomes professor now at CSU, never would have expected that resiliency of life before this project, even with her microbiology background. "We can inject a handful of cells into an environment that's so foreign and almost toxic, and life finds ways to proliferate and do so very well," says Wrighton. "I've been really astounded by that."

Giant bioreactors

The scientists know the microbes originate from the surface, but they are still figuring out from exactly where in the engineering process they are coming, according to Wrighton. They do know the subsurface microbial community looks significantly different from the freshwater input.

Certain organisms that make up less than one percent of the microbial community at the surface can bloom in the unique subsurface environment, says Wrighton, and become over 80 percent of the community.

In an earlier *Nature Microbiology* paper, the research team found only 31 different genomes existed in the fluids produced up to nearly a year after fracking. Those findings also indicated the streamlined genomes were similar in wells of two different shales – Marcellus and Utica –

located hundreds of miles apart in the Appalachian Basin.

These microbial communities are far less diverse than those in most other known systems. The human gut, for instance, contains about 2,000 unique genomes.

That simplicity enables the researchers to tease out the processes and interactions happening in the system and decipher how the microbial community persists under the harsh conditions.

For example, the researchers tracked an amino acid derivative, called glycine betaine, produced by microbes that consume chemical additives in the fluid. The compound serves both as protection against high salinity for some microbes and as an energy source for others. The bacteria that feed on it generate food for other bacteria, and the cycle goes on and on.

Deep underground, resources are limited and not replenished, so the microbes have to adapt to what's available.



Graduate student Kaela Amundson and Michael Wilkins filtering produced hydraulic fracturing fluids in the field. Credit: Kayla Borton, CSU

These cycles and processes are harder to resolve in more diverse microbial communities, such as wetlands and soils, says Wilkins. While it is difficult to track how the dynamics of those complex systems change over time, characterizing the relatively easy fractured shale communities can answer some of the important questions.

Hand in hand

To help tease out these mechanisms, researchers recreate the shale microbial community in reactors in the lab, as described in a *PNAS*

publication. There, they can test their theories by applying stress or changes that aren't feasible at the deep underground field sites. The technique goes hand in hand with genomic sequencing, says Wilkins.

The researchers can then confirm their lab findings with modeling of the field-collected data. Collecting measurements at multiple levels of omics – including metabolomics and proteomics – strengthens the results. Fortunately, EMSL has both the expertise and capabilities to support that.

By collaborating and looking at questions from different levels, says Hoyt, "you get a much more powerful story."

That approach helped the research team to dive even deeper in the most recent study in *Nature Microbiology*, where they investigated the interactions between host bacteria and their much smaller viruses in the shale communities. Despite the low diversity of the bacteria, the viral genome results showed a high variety of viruses.

The team also found the virus can sense when their hosts are stressed, and they will then break into the cell walls and release the contained metabolites to the surrounding fluids. The remaining neighboring bacteria subsequently consume those metabolites. Destroying one microbe helps support the overall community.

Colonizing knowledge

Now the research team is expanding to other shales from across North America and seeing how the [microbial communities](#) in those fractured shale communities compare to the ones they studied in the Appalachian Basin. The preliminary results, says Wrighton, indicate that while not all the microbial species are the same, the community processes are.

These processes can have consequences on the bottom line of the fracking industry, which supplies a significant amount of energy used on the Eastern seaboard. Some processes can produce more methane gas, while others create byproducts like sulfides that can "sour gas" and corrode the equipment. With knowledge of these engineered worlds, the industry might be able to control the communities in the industry's favor over the long term.

Despite the uniqueness of this particular microbial ecosystem, it can help advance basic science, because it's so tractable and manageable. For example, Wrighton says this system is a good place to start testing out some of the questions researchers have for how probiotics work in the human body.

But Wilkins suggests an application in the beyond: "This could be a great [model system](#) for how microbial life might persist in subsurface, highly saline ecosystems on other planets."

More information: Rebecca A. Daly et al. Viruses control dominant bacteria colonizing the terrestrial deep biosphere after hydraulic fracturing, *Nature Microbiology* (2018). [DOI: 10.1038/s41564-018-0312-6](https://doi.org/10.1038/s41564-018-0312-6)

Mikayla A. Borton et al. Coupled laboratory and field investigations resolve microbial interactions that underpin persistence in hydraulically fractured shales, *Proceedings of the National Academy of Sciences* (2018). [DOI: 10.1073/pnas.1800155115](https://doi.org/10.1073/pnas.1800155115)

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