STEFAN GREEN/XMP

Researchers are looking for microbes in soil samples from this flaming gas crater in Turkmenistan, known as the Door to Hell.

STUDYING LIFE AT THE EXTREMES

Researchers have invented methods to study microbes that thrive in the world's most inhospitable environments. **By Amber Dance**

Microbes cling to life in some of Earth's most extreme environments, from toxic hot springs to high-altitude deserts. These 'extremophiles' include organisms that can survive near-boiling heat or near-freezing cold, high pressure or high salt, as well as environments steeped in acids, alkalis, metals or radioactivity.

Coercing these organisms to live in laboratories creates many challenges. Nonetheless, papers published on extremophiles have doubled in the past decade. Some scientists are drawn to the novelty of the organisms, searching for ones that are undescribed or that might harbour useful enzymes for industrial processes or antibiotics to save lives. Others simply find that the best organism for their scientific questions happens to have extreme preferences.

It's a circumstance that has forced researchers who study extremophiles to invent new laboratory methods for handling them. To identify, culture, genetically manipulate and observe extremophiles, researchers often tweak the methods used in more run-of-the-mill organisms. Whereas some techniques can be easily transferred — from one thermophile to other heat-lovers, say — others have to be adapted for each new organism.

"Each extremophile is going to have its own set of challenges," says Jocelyne DiRuggiero, a microbiologist at Johns Hopkins University in Baltimore, Maryland. "Every time you think about doing something, you have to think, how am I going to adapt this for my organism?"

Growing at extremes

In 2014, Scott Tighe, a microbiologist at the University of Vermont in Burlington, gathered international collaborators for an ongoing initiative known as the Extreme Microbiome Project (XMP). The researchers hope to find genes that might indicate how extremophiles survive and whether they might make compounds that could work as antibiotics.

In their search for new microbes to analyse, the scientists have travelled to extreme locales, including the toxic hot springs of Ethiopia's Danakil Depression, which are loaded with salt, acids and heavy metals, and a flaming gas crater in Turkmenistan.

But their main challenges arose back in the lab: "Massive hurdles, actually," says Tighe. Microbes tough enough to withstand extreme conditions resisted scientists' attempts to break them open and recover their DNA. So Tighe and the XMP team developed a six-enzyme cocktail — now commercially available as MetaPolyzyme — to break down any cell surface they might come across, adding detergents and organic solvents to collect nucleic acid on magnetic beads[1]. "It got to be a fairly exotic DNA-extraction technique," says Tighe. Thus armed, the researchers are working through a

freezer full of samples, says Tighe.

Studying living extremophiles in the lab creates challenges, too. DiRuggiero's group, for example, works with salt-loving halophiles, chipped out of rocks from Chile's high-altitude Atacama Desert. Some such organisms are easy to cultivate, she says: just add salt. DiRuggiero has also worked with anaerobic hyperthermophiles, which grow at high heat and in the absence of oxygen. To culture them, she used heat-resistant, NASA-made culture jars in an incubator set to 95 °C – so toasty, the team jokingly called it the "pizza oven". Standard agar in plastic Petri dishes would melt, so scientists turn to glass dishes packed with a gellan-gum derivative called Gelrite, which can withstand the heat.

Manipulating genes

Altering extremophiles' genes can require extra effort. The tools and techniques that work in lab favourites such as *Escherichia coli* – including the plasmids that are used to transfer genetic material, methods to insert that material into new microbes and compounds to select microorganisms that have successfully integrated new genes – are frequently ill-suited to high levels of salt, roasting temperatures and other extreme environments.

Scientists often create the genes they want to use in *E. coli*, isolate the relevant plasmids, and then transfer them into the extremophiles. Some extremophiles can take up nucleic acids from the surrounding media, says Carrie Eckert, a synthetic biologist at the University of Colorado Boulder and the National Renewable Energy Laboratory in Golden, Colorado. But if that doesn't work, she recommends electroporation, a pulse of electricity to open up holes in the cells' membranes.

Another tip, she says, is to determine the methylation patterns on the extremophile's genome, through sequencing[2]. That's important because microorganisms will often destroy nucleic acids that have the 'wrong' pattern, to protect themselves from invaders. Scientists are learning to modify those methylation systems in *E. coli* to make new DNA match that of the host[3].

Once they've provided correctly methylated DNA, scientists must pick out the extremophiles that take it up. To investigate the genes they're interested in, microbiologists often insert them into plasmids that contain genes for antibiotic resistance; colonies that grow on media containing that antibiotic have taken up the genes. But many antibiotics fail to function under extreme conditions. An alternative selection tool is an organism's ability to grow (or not) without certain nutrients – this tends to be less sensitive to unusual conditions.

Researchers have also adapted gene editing for extremophiles. Eckert's team developed a two-step strategy to edit the genome of the thermophile *Clostridium thermocellum*[4]. They borrowed a gene-swapping system from another thermophile to introduce the desired sequence into *C. thermocellum*. At the same time, they changed nearby sites that normally allow a CRISPR–Cas system to recognize and cut the DNA, rendering them invisible to CRISPR. Then, they applied CRISPR–Cas to slice any unmodified genes, eliminating unedited microbes.

"The main thing is, just try. The 'extremophily' is often not as much of a barrier as you first might think."

The strategy has up to 94% efficiency, and should work for other thermophiles, too, says Eckert. The necessary microbial strains are available from New England Biolabs and the American Type Culture Collection, and Eckert is working to add the plasmids to the Addgene repository.

Live on camera

Microscopes are also getting a makeover to investigate extremophile cell biology.

Buzz Baum, a cell biologist at the Medical Research Council Laboratory for Molecular Biology in Cambridge, UK, is interested in cell division in the thermophile and acidophile *Sulfolobus acidocaldarius*. But live cultures cool as quickly as a cup of tea on the microscope stage, says Baum, and the microbes go into suspended animation. "We spent many years failing."

The team decided to borrow a technique used in polymerase chain reaction machines, to warm the cultures from the top as well as the bottom. They recruited a lab member's father and brother, both aerospace engineers, to fabricate a chamber out of aircraft aluminium. The researchers have published diagrams for this 'Sulfoscope'[5], but any double-heated chamber should do, says Baum. The system allows the team to investigate proteins that maintain symmetrical cell division.

Halophiles, too, are tricky to work with under the microscope. They lack the rigid cell walls found in bacteria, and survive by maintaining the same osmotic pressure as their environment, giving them all the rigidity of a limp balloon. That means they're easily deformed when sandwiched between a microscope slide and coverslip, making it difficult to study the size and shape of the cells.

Ye-Jin Eun encountered this problem during a postdoc at Harvard University in Cambridge, Massachusetts, while investigating how the salt-loving archaeon *Halobacterium salinarum* controls its size. The organisms, which are shaped like rods in liquid cultures, deformed into bizarre polygonal shapes or amorphous blobs when she used a soft agar pad to hold the cells in place for microscopy. "We didn't think it would be so squishable," says Eun, now a principal data scientist at Janssen in Titusville, New Jersey. A microfluidics device called the CellASIC ONIX, from Millipore Sigma in Burlington, Massachusetts, pins microbes using soft polydimethylsiloxane, but the material proved toxic to Eun's cells.

She finally succeeded by fabricating tiny agarose chambers to confine the cells gently. At last, Eun could see that the archaeon maintains its size in much the same way as bacteria do, with each newborn cell adding a consistent length to its rod before dividing again[6].

Get your glow on

Microscopists must also find ways to label proteins of interest under extreme conditions.

For example, an international team faced a challenge with a halophile called *Haloferax volcanii*, found in the Dead Sea, because it makes a pigment that naturally fluoresces. That made it tricky to use fluorescent protein tags for tracking single molecules, the group reported in a preprint in July[7]. So, first, the team deleted a gene involved in synthesizing that pigment, creating colourless but otherwise normal microbes.

Then, the researchers tackled the genetic milieu of *H. volcanii*. Like many halophiles, *H. volcanii* has DNA containing a relatively high proportion of guanine and cytosine bases, so its codons – the triplet sequences that encode amino acids – also tend to use those bases. Furthermore, genes that use the same codons will be expressed at higher levels. So the team optimized the codons used for fluorescent proteins that work in non-extreme organisms to match the extremophile's preferences.

"Most of them worked," says co-author Iain Duggin, a molecular microbiologist at the University of Technology Sydney in Australia. Versions of the crimson-coloured protein mCherry and the green-to-red photoswitchable tag Dendra2 were especially useful for single-molecule tracking. "We're really excited about how this can be applied to studies of cell division and shape and the cytoskeleton," says Duggin.

Indeed, with perseverance, scientists interested in extremophiles can make impossible-seeming experiments work. "The main thing is, just try," says Duggin. "The 'extremophily', if you will, is often not as much of a barrier as you first might think."

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