Engineers find a way to protect microbes from extreme conditions

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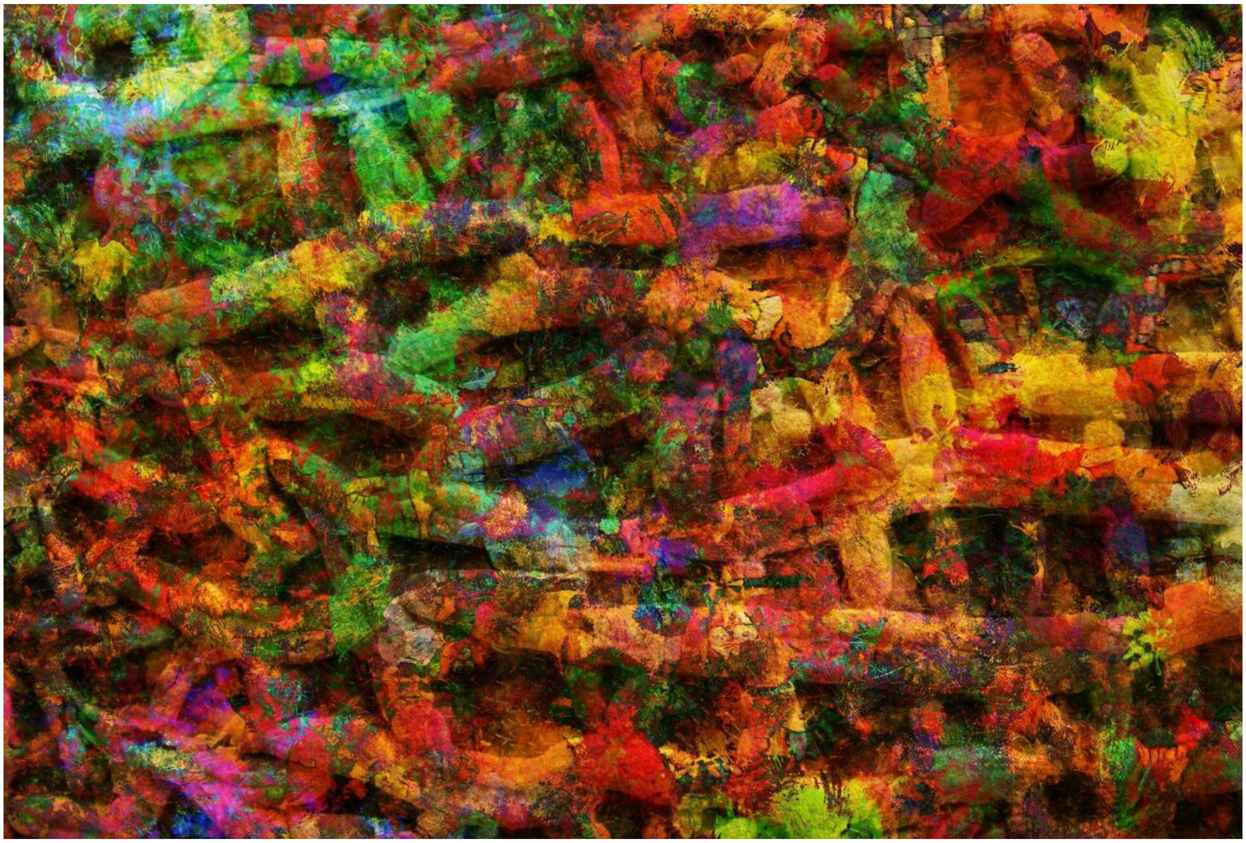

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Microbes that are used for health, agricultural, or other applications need to be able to withstand extreme conditions, and ideally the manufacturing processes used to make tablets for long-term storage.

MIT researchers have now developed a new way to make microbes hardy enough to withstand these extreme conditions.

Their method involves mixing bacteria with food and drug additives from a list of compounds that the FDA classifies as "generally regarded as safe." The researchers identified formulations that help to stabilize several different types of microbes, including yeast and bacteria, and they showed that these formulations could withstand high temperatures, radiation, and industrial processing that can damage unprotected microbes.

In an even more extreme test, some of the microbes recently returned from a trip to the International Space Station, coordinated by Space Center Houston Manager of Science and Research Phyllis Friello, and the researchers are now analyzing how well the microbes were able to withstand those conditions.

"What this project was about is stabilizing organisms for extreme conditions. We're really thinking about a broad set of applications, whether it's missions to space, human applications, or agricultural uses," says Giovanni Traverso, an associate professor of mechanical engineering at MIT, a gastroenterologist at Brigham and Women's Hospital, and the senior author of the study.

Miguel Jimenez, a former MIT research scientist who is now an assistant professor of biomedical engineering at Boston University, is the lead author of the paper, which appears in *Nature Materials*.

Surviving extreme conditions

About six years ago, Traverso's lab began working on new approaches to make helpful bacteria such as probiotics and microbial therapeutics more resilient. As a starting point, the researchers analyzed 13 commercially available probiotics and found that six of these products did not contain as many live bacteria as the label indicated.

"What we found was that—perhaps not surprisingly—there is a difference, and it can be significant," Traverso says. "So then the next question was, given this, what can we do to help the situation?"

For their experiments, the researchers chose four different microbes to focus on: three bacteria and one yeast. These microbes are Escherichia coli Nissle 1917, a probiotic; Ensifer meliloti, a bacterium that can fix nitrogen in soil to support plant growth; Lactobacillus plantarum, a bacterium used to ferment food products; and the yeast Saccharomyces boulardii, which is also used as a probiotic.

When microbes are used for medical or agricultural applications, they are usually dried into a powder through a process called lyophilization. However, they can not normally be made into more useful forms such as a tablet or pill because this process requires exposure to an organic solvent, which can be toxic to the bacteria. The MIT team set out to find additives that could improve the microbes' ability to survive this kind of processing.

"We developed a workflow where we can take materials from the 'generally regarded as safe' materials list from the FDA, and mix and match those with bacteria and ask, are there ingredients that enhance the stability of the bacteria during the lyophilization process?" Traverso says.

Their setup allows them to mix microbes with one of about 100 different ingredients and then grow them to see which survive the best when stored at room temperature for 30 days. These experiments revealed different ingredients, mostly sugars and peptides, that worked best for each species of microbe.

The researchers then picked one of the microbes, E. coli Nissle 1917, for further optimization. This probiotic has been used to treat "traveler's diarrhea," a condition caused by drinking water contaminated with harmful bacteria. The researchers found that if they combined caffeine or yeast extract with a sugar called melibiose, they could create a very stable formulation of E. coli Nissle 1917.

This mixture, which the researchers called formulation D, allowed survival rates greater than 10% after the microbes were stored for six months at 37 degrees Celsius, while a commercially available formulation of E. coli Nissle 1917 lost all viability after only 11 days under those conditions.

Formulation D was also able to withstand much higher levels of ionizing radiation, up to 1,000 grays. (The typical radiation dose on Earth is about 15 micrograys per day, and in space, it's about 200 micrograys per day.)

The researchers don't know exactly how their formulations protect bacteria, but they hypothesize that the additives may help to stabilize the bacterial cell membranes during rehydration.

Stress tests

The researchers then showed that these microbes can not only survive harsh conditions, they also maintain their function after these exposures. After Ensifer meliloti were exposed to temperatures up to 50 degrees Celsius, the researchers found that they were still able to form symbiotic nodules on plant roots and convert nitrogen to ammonia.

They also found that their formulation of E. coli Nissle 1917 was able to inhibit the growth of Shigella flexneri, one of the leading causes of diarrhea-associated deaths in low- and middle-income countries, when the microbes were grown together in a lab dish.

Last year, several strains of these extremophile microbes were sent to the International Space Station, which Jimenez describes as "the ultimate stress test."

"Even just the shipping on Earth to the preflight validation, and storage until flight are part of this test, with no temperature control along the way," he says.

The samples recently returned to Earth, and Jimenez's lab is now analyzing them. He plans to compare samples that were kept inside the ISS to others that were bolted to the outside of the station, as well as control samples that remained on Earth.

Other authors of the paper include Johanna L'Heureux, Emily Kolaya, Gary Liu, Kyle Martin, Husna Ellis, Alfred Dao, Margaret Yang, Zachary Villaverde, Afeefah Khazi-Syed, Qinhao Cao, Niora Fabian, Joshua Jenkins, Nina Fitzgerald, Christina Karavasili, Benjamin Muller, and James Byrne.

More information: Synthetic extremophiles via species-specific formulations improve microbial therapeutics, *Nature Materials* (2024). DOI: 10.1038/s41563-024-01937-6