DNA-barcoded microbial spores can trace origin of objects, agricultural products

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Every year, an estimated 48 million Americans get sick from foodborne illnesses, resulting in some 128,000 hospitalizations and 3,000 deaths, according to the U.S. Centers for Disease Control and Prevention. This public health problem is compounded by billions in economic damage from product recalls, highlighting the need to rapidly and accurately determine the sources of foodborne illnesses.

With the increasing complexity of global supply chains for the myriad foods available to consumers, however, the task of tracing the exact origin of contaminated items can be difficult.

In a novel solution that can help determine the origin of agricultural products and other goods, Harvard Medical School scientists have developed a DNA-barcoded microbial system that can be used to label objects in an inexpensive, scalable and reliable manner.

Reporting in *Science* on June 4, the research team describes how synthetic microbial spores can be safely introduced onto objects and surfaces at a point of origin, such as a field or manufacturing plant, and be detected and identified months later.

The spores are derived from baker's yeast and a common bacterial strain used in a wide variety of applications, such as probiotic dietary supplements, and designed to be incapable of growing in the wild to prevent adverse ecological effects.

"Spores are in many ways an old-school solution and have been safely sprayed onto agricultural goods as soil inoculants or biological pesticides for decades. We just added a small DNA sequence we can amplify and detect," said study corresponding author Michael Springer, associate professor of systems biology in the Blavatnik Institute at HMS.

"We also worked hard to make sure this system is safe, using commonplace microbial strains and building in multiple levels of control," Springer added. "We hope it can be used to help solve problems that have enormous public health and economic implications."

In recent years, scientists have learned a great deal about the interactions between microbes and their environments. Studies show that microbial communities in homes, on cell phones, on human bodies and more have unique compositions, similar to fingerprints. Attempts to use microbial fingerprints to identify provenance can be time consuming and are not easily scaled, however.

The use of custom-synthesized DNA sequences as barcodes has been shown in principle to be effective for labeling food and other items. To be widely useful, DNA barcodes must be produced cheaply in large volumes, persist on objects in highly variable environments, and able to be reliably and rapidly decoded—hurdles that have thus far not been overcome because DNA is fragile.

Heavy-duty packaging

In their study, Springer and colleagues set out to determine if DNA barcodes packaged within microbial spores, which can be sprayed onto crops and identified months later, could help solve these challenges.

Many microorganisms, including bacteria, yeasts and algae, form spores in response to harsh environmental conditions. Analogous to seeds, spores allow microorganisms to remain dormant for extraordinarily long periods and survive extreme conditions such as high temperatures, drought and UV radiation.

The research team created custom-made DNA sequences that they integrated into the genomes of the spores of two microorganisms—Saccharomyces cerevisiae, also known as baker's yeast, and Bacillus subtilis, a common and widespread bacterium that has numerous commercial uses, including as a dietary probiotic, a soil inoculant and a fermenting agent in certain foods. These spores can be cheaply grown in the lab in large numbers.

The synthetic DNA sequences are short and do not code for any protein product, and are thus biologically inert. Inserted into the genome in tandem, the sequences are designed so that billions of unique barcodes can be created.

The team also ensured that DNA-barcoded spores could not multiply, grow and spread in the wild. They did so by using microbial strains that require specific nutritional supplementation and by deleting genes required for the spores to germinate and grow. Experiments involving from hundreds of millions to more than a trillion of the modified spores confirmed that they are unable to form colonies.

To read the DNA barcodes, the researchers used an inexpensive CRISPR-based tool that can detect the presence of a genetic target rapidly and with high sensitivity. The technology, called SHERLOCK, was developed at the Broad Institute of MIT and Harvard, in a collaboration led by institute members James Collins and Feng Zhang.

"Spores can survive in the wild for an extremely long time and are a great medium for us to incorporate DNA barcodes into," said study co-first author Jason Qian, a graduate student in systems biology at HMS. "Identifying the barcodes is straightforward, using a plate reader and an orange plastic filter on a cell phone camera. We don't envision any challenges for field deployability."

Real world

The team examined the efficacy of their barcoded microbial spore system through a variety of experiments.

They grew plants in the laboratory and sprayed each plant with different barcoded spores. A week after inoculation, a leaf and a soil sample from each pot were harvested. The spores were readily detected, and even when the leaves were mixed together, the team could identify which pot each leaf came from.

When sprayed onto grass outside and exposed to natural weather for several months, spores remained detectable, with minimal spread outside the inoculated region. On environments such as sand, soil, carpet and wood, the spores survived for months with no loss over time, and they were identified after disturbances such as vacuuming, sweeping and simulated wind and rain.

Spores are very likely to persist through the conditions of a real-world supply chain, according to the researchers. As a proof-of-principle, they tested dozens of store-bought produce items for the presence of spores of Bacillus thuringiensis (Bt), a bacterial species that is widely used as a pesticide. They correctly identified all Bt-positive and Bt-negative plants.

In additional experiments, the team built a 100-square-meter (~1000 square feet) indoor sandpit and found that the spread of spores was minimal after months of simulated wind, rain and physical disturbances.

They also confirmed that spores can be transferred onto objects from the environment. Spores were readily identified on the shoes of people who walked through the sandpit, even after walking for several hours on surfaces that were never exposed to the spores. However, the spores could not be detected on these surfaces, suggesting that objects retain the spores without significant spread.

This characteristic, the team noted, could allow spores to be used to determine whether an object has passed through an inoculated area. They tested this by dividing the sandpit into grids, each labeled with up to four different barcoded spores. Individuals and a remote-control car then navigated the sandpit.

They found that they could identify the specific grids that the objects passed through with minimal false positives or negatives, suggesting a possible application as a complementary tool for forensics or law enforcement.

The team also considered potential privacy implications, noting that existing technologies such as UV dyes, cell phone tracking and facial recognition are already widely used but remain controversial.

"As scientists, our charge is to solve scientific challenges, but at the same time we want to make sure that we acknowledge broader societal implications," Springer said. "We believe the barcoded spores are best suited for farming and industrial applications and would be ineffective for human surveillance." Regardless, the use and adoption of this technology should be done with a consideration of ethics and privacy concerns, the study authors said.

The researchers are now exploring ways to improve the system, including engineering potential kill-switch mechanisms into the spores, finding ways to limit propagation and examining if the spores can be used to provide temporal information about location history.

"Outbreaks of harmful foodborne pathogens such as listeria, salmonella and E. coli occur naturally and frequently," Springer said. "Simple, safe synthetic biology tools and knowledge of basic biology allow us to create things that have a lot of potential in solving real world safety issues."

More information: J. Qian el al., "Barcoded microbial system for high-resolution object provenance," *Science* (2020).
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