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Tiny barcodes for a global food chain

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Determining the origin of the foods we eat is harder than ever in our globalized world, yet in the case of food borne illness this answer could save lives and money. By harnessing microbial spores, we developed a technique using microscopic DNA barcodes to determine the origin of objects in a way that is safe, scalable, and durable to be used in real-world settings.

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Do you know where your food comes from? How would you find out? Every year, an estimated 48 million Americans get sick from foodborne illnesses. Traditionally, when an outbreak occurs, such as the multi-state E. coli outbreak in romaine lettuce in 2018, the source of contamination is determined by comparing meal histories of patients - an imperfect process. This often leads to widespread product recalls which cost money and lose customers. We asked whether there was a better way to label the origin of an object, or record its path through locations of interest (for example processing areas). This is known as the object provenance problem.

Recent work showed objects gradually accumulate the naturally occurring microbes present in their environment, known as the microbiome. In theory, by identifying the collection of microbes on an object, and linking those microbes to the locations they came from, you could reconstruct the provenance of an object. However, this would require lots of expensive mapping of the microbiome for every location of interest. Instead, we envisioned making synthetic microbe-inspired tags which can be deliberately introduced onto areas or objects of interest, which provides a unique readout for the provenance of an object. Like a barcode on a package you see at the store, only at a microscopic scale.

Inspired by nature, we decided to use microbial spores, which like plant seeds, are designed to survive and persist in the environment for long periods without growth, even in extreme conditions like high temperatures. We worked with handicapped strains of two common spore forming microbes: *Saccharomyces cerevisiae* (the yeast used in baking and brewing), and *Bacillus subtilis* (a common soil bacterium that is non-harmful to humans). We introduced short DNA barcodes into the genome of these organisms, so that each batch of spores has a unique barcode that can later be read out by detection tests. The test we used to detect the barcodes is called SHERLOCK; it emits light in the presence of specific DNA barcodes which can then simply be imaged using a smartphone camera.

We then showed how barcoded microbial spores could be used in real-world applications. First, we grew batches of spores labelled with different barcodes, then killed them to prevent growth. We then sprayed these spores onto a surface, thus labelling the surface - and objects that later contact it - with our microscopic barcoded microbial spores. We showed that spores persisted without being degraded for months on surfaces like sand, soil, wood, or carpet, both in indoor and outdoor environments. Furthermore, we could recover these spores from shoes and other objects that travel through labelled areas and use them to reconstruct an item's trajectory.

Our most exciting demonstration was related to combatting food-borne illness. Spores of *Bacillus thuringensis* are commonly sprayed on crops as a natural biocide in agriculture. Modelling this process using our barcoded microbial spores, we sprayed different plants with different barcoded spores. Weeks later, after harvesting the leaves, we were able to link each leaf back to its plant by identifying the barcode. Excitingly, this worked even after mixing leaves together or simulated cooking! To see whether spores can persist all the way through the food supply chain, rather than using our barcoded microbial spores, we looked for DNA from *Bacillus thuringensis* on produce from grocery stores and local farms. Indeed, we detected *Bacillus thuringensis* spores on a variety of produce. Therefore, if we introduced our DNA barcodes into *Bacillus thuringensis*, and sprayed each farm using a different barcoded strain, a simple detection test would be all that is needed to identify the source of any foodborne illness - down to the exact field, farm, or processing facilities that the food came from!

Broadly, our technology provides a robust, scalable, safe, and straightforward solution to the object provenance problem. However, before barcoded microbial spores go into widespread use, there are certain remaining questions, both practical and ethical. How do barcoded microbial spores perform in real-world supply chains that may be far more complex than our experiments? What is the best way to regulate the use of barcoded microbial spores?

With more complex synthetic biology techniques, other aspects of an object's history could be made detectable. Wondering whether your food was stored at an inappropriate temperature? Exposed to pollutants? Perhaps these questions could be answered with "next-generation" spores that record information about their surroundings over time.