Growing unknown microbes one by one

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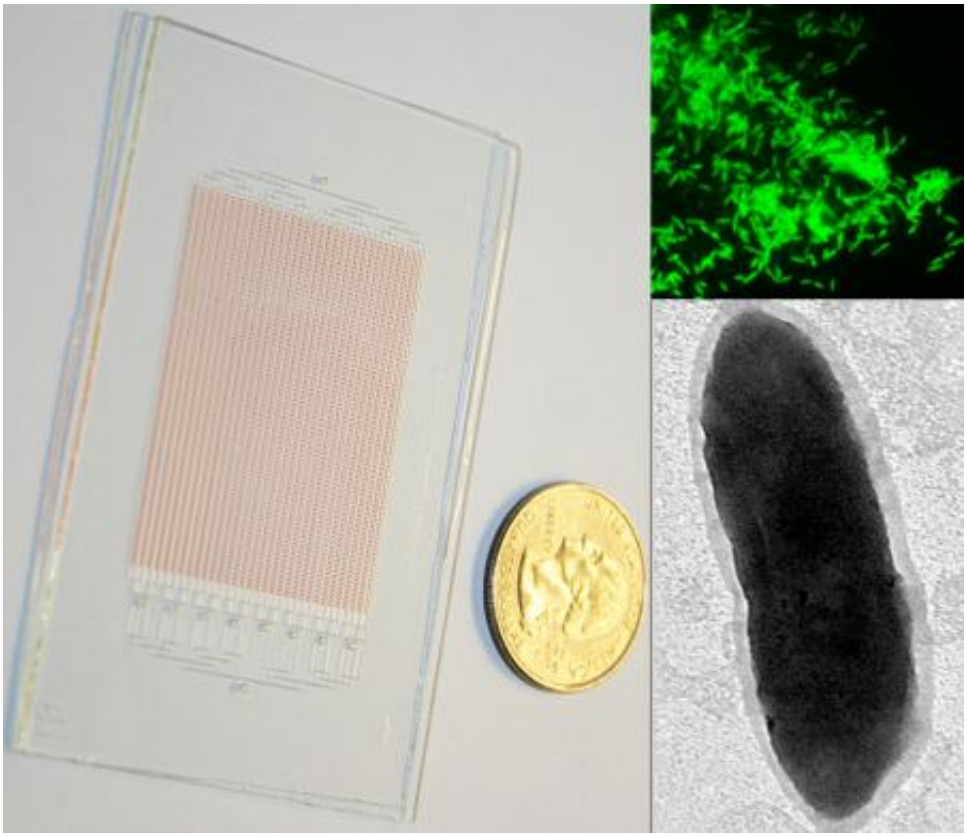

Photograph of a glass SlipChip for growing microbes, shown next to a US quarter (left). Fluorescent in situ hybridization image of the target organism (right, top). Transmission electron microscopy image of a single cell of the target organism (right, bottom). Credit: Liang Ma (Ismagilov group) and Roland Hatzenpichler/Caltech

(Phys.org) —Trillions of bacteria live in and on the human body; a few species can make us sick, but many others keep us healthy by boosting

digestion and preventing inflammation. Although there's plenty of evidence that these microbes play a collective role in human health, we still know very little about most of the individual bacterial species that make up these communities. Employing the use of a specially designed glass chip with tiny compartments, Caltech researchers now provide a way to target and grow specific microbes from the human gut—a key step in understanding which bacteria are helpful to human health and which are harmful.

The work was published the week of June 23 in the *Proceedings of the National Academy of Sciences.*

Although a few bacterial species are easy to grow in the laboratory, needing only a warm environment and plenty of food to multiply, most species that grow in and on the human body have never been successfully grown in lab conditions. It's difficult to recreate the complexity of the microbiome—the entire human microbial community—in one small plate (a lidded dish with nutrients used to grow microbes), says Rustem Ismagilov, Ethel Wilson Bowles and Robert Bowles Professor of Chemistry and Chemical Engineering at Caltech.

There are thousands of species of microbes in one sample from the human gut, Ismagilov says, "but when you grow them all together in the lab, the faster-growing bacteria will take over the plate and the slow-growing ones don't have a chance—leading to very little diversity in the grown sample." Finding slow-growing microbes of interest is like finding a needle in a haystack, he says, but his group wanted to work out a way to "just grow the needle without growing the hay."

To do this, Liang Ma, a postdoctoral scholar in Ismagilov's lab, developed a way to isolate and cultivate individual bacterial species of interest. He and his colleagues began by looking for bacterial species that

contained a set of specific genetic sequences. The targeted gene sequences belong to organisms on the list of "Most Wanted" microbes—a list developed by the National Institutes of Health (NIH) Human Microbiome Project. The microbes carrying these genetic sequences are found abundantly in and on the human body, but have been difficult to grow in the lab.

To grow these elusive microbes, the Caltech researchers turned to SlipChip, a microfluidic device previously developed in Ismagilov's lab. SlipChip is made up of two glass slides, each the size of a credit card, that have tiny etched grooves which become channels when the grooved surfaces are stacked atop one another. When a sample—say, a jumbled-up assortment of bacteria species collected from a colonoscopy biopsy—is added to the interconnected channels of the SlipChip, a single "slip" of the top chip will turn the channels into individual wells, with each well ideally holding a single microbe. Once sequestered in an isolated well, each individual bacterium can divide and grow without having to compete for resources with other types of faster-growing microbes.

The researchers then needed to determine which compartment of the SlipChip contained a colony of the target bacterium—which is not a simple task, says Ismagilov. "It's a Catch-22—you have to kill the organism in order to find its DNA sequence and figure out what it is, but you want a live organism at the end of the day, so that you can grow and study this new microbe," he says. "Liang solves this in a really clever way; he grows a compartment full of his target microbe in the SlipChip, then he splits the compartment in half. One half contains the live organism and the other half is sacrificed for its DNA to confirm that the sequence is that of the target microbe."

The method of creating two halves in each well in the SlipChip will be published separately in an upcoming issue of the journal *Integrative*

Biology.

To validate the new methodology, the researchers isolated one specific bacterium from the Human Microbiome Project's "Most Wanted" list. The investigators used the SlipChip to grow this bacterium in a tiny volume of the washing fluid that was used to collect the gut bacteria sample from a volunteer. Since bacteria often depend on nutrients and signals from the extracellular environment to support growth, the substances from this fluid were used to recreate this environment within the tiny SlipChip compartment—a key to successfully growing the difficult organism in the lab.

After growing a pure culture of the previously unidentified bacterium, Ismagilov and his colleagues obtained enough genetic material to sequence a high-quality draft genome of the organism. Although a genomic sequence of the new organism is a useful tool, further studies are needed to learn how this species of microbe is involved in human health, Ismagilov says.

In the future, the new SlipChip technique may be used to isolate additional previously uncultured microbes, allowing researchers to focus their efforts on important targets, such as those that may be relevant to energy applications and the production of probiotics. The technique, says Ismagilov, allows researchers to target specific microbes in a way that was not previously possible.

The paper is titled "Gene-targeted microfluidic cultivation validated by isolation of a gut bacterium listed in Human Microbiome Project's Most Wanted taxa." In addition to Liang and Ismagilov, other coauthors include, from Caltech, associate scientist Mikhail A. Karymov, graduate student Jungwoo Kim, and postdoctoral scholar Roland Hatzenpichler, and, from the University of Chicago department of medicine, Nathanial Hubert, Ira M. Hanan, and Eugene B. Chang. The work was funded by

NIH's National Human Genome Research Institute. Microfluidic technologies developed by Ismagilov's group have been licensed to Emerald BioStructures, RanDance Technologies, and SlipChip Corporation, of which Ismagilov is a cofounder.

More information: Gene-targeted microfluidic cultivation validated by isolation of a gut bacterium listed in Human Microbiome Project's Most Wanted taxa , *PNAS*, www.pnas.org/cgi/doi/10.1073/pnas.1404753111

"Individually addressable arrays of replica microbial cultures enabled by splitting SlipChips." Ma, Liang and Datta, Sujit S. and Karymov, Mikhail A. and Pan, Qichao and Begolo, Stefano and Ismagilov, Rustem F. (2014) Individually addressable arrays of replica microbial cultures enabled by splitting SlipChips. *Integrative Biology* . ISSN 1757-9694. resolver.caltech.edu/CaltechAU … S:20140620-180156638

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