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Probing Biology's Dark Matter

A typical human mouth teems with as many as 700 different species of microbes. A handful of these have been specifically implicated in promoting gum disease, dental cavities, and bad breath, but for the most part, the make-up of this complex ecosystem and its impact on human health remain largely unexplored. A new device created by Howard Hughes Medical Institute (HHMI) researchers, however, may make some of the most reclusive members of this and other microscopic communities much more accessible for laboratory study.

The vast majority of microbes are notoriously resistant to growing in laboratory cultures because they are so intricately linked to their own unique ecosystems. Microbiologists have coaxed less than one percent of the bacterial species that inhabit natural environments into growing in culture. But a microfluidics device created by Howard Hughes Medical Institute investigator Stephen R. Quake and colleagues at Stanford University—an intricate system of miniscule valves and chambers—may help scientists who want to identify and characterize new microbes circumvent the need to culture them at all.

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— Stephen R. Quake

Research on the device published in the July 9, 2007, issue of the *Proceedings of the National Academy of Sciences (PNAS)* has far-reaching implications for the rapidly developing field of microbial ecology, as well as advancing microfluidics technologies, which could do for biology what silicon chips did for electronics. Quake and his colleagues have already used the device to analyze a rare bacteria found in the human mouth, using just a single cell.

Various methods have given scientists a glimpse of the profound diversity that characterizes different microbial worlds. One approach is to look for variations in the sequence of a specific gene found in all microorganisms; another is a complete inventory of all the pooled genes in a microbial

community. These types of studies, however, yield few insights into the character of individual members of a microbial ecosystem, leaving most species almost entirely enigmatic.

Those unstudied organisms are biology's dark matter, Quake says. Like the dark matter that astronomers can only infer must exist in the universe, these organisms have never been studied directly. Quake and his colleagues hope their new technology will change that.

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Quake's research lies at the nexus of physics, biology, and biotechnology. His microfluidics chips, which he designs to tackle problems in fields including structural genomics, systems biology, microbial ecology, and synthetic chemistry, are akin to having a fully automated laboratory on a postage stamp-sized wafer. Remember the early days of electronics with all of those big vacuum tubes and wires? Next came the transistor and finally the silicon chip, which dramatically revolutionized computers and modern electronics. Microfluidics is following the track of silicon chips and promises to revolutionize biology in the same manner.

The microfluidic chip designed by Quake and his colleagues for the current study is equipped with tiny chambers and valves that allow researchers to isolate microbes at the nanoliter scale. Because each microbe is isolated in a vanishingly small volume of liquid, the concentration of its genetic material within that solution is actually quite high - meaning Quake and his colleagues can easily amplify and analyze the genome of an individual cell, eliminating the need to persuade the organism to multiply in a laboratory culture. The chip offers the potential to discover untold new species of microbes lurking within deep sea vents, ordinary dirt, toxic sludge, or virtually any environment.

To demonstrate the power of the new device, the scientists first used it to target a possible new phylum, of which one member is a rod-shaped bacterium that live between the gums and teeth of humans. The candidate phylum, called TM7, has no cultivated or sequenced members. The scientists demonstrated that they could inject a solution containing multiple types of microbes into a chip, and manipulate tiny valves to direct individual rod-shaped bacteria into miniature chambers. Once individual microbes were isolated, the researchers could extract the DNA and amplify it using routine methods.

In this way, the researchers were able to sequence and assemble more than 1,000 genes, providing insight into the physiology of this previously unstudied group of bacteria. Most TM7 genes, they found, had remarkably little similarity to genes in known bacterial groups. But some of the genes hinted at interesting aspects of the organism's biology, such as an unusual gliding motion that groups of TM7 bacteria might use to get around, and a gene shared with bacteria known to cause chronic inflammation.

Just as importantly, the researchers say, they have demonstrated the success of their new technology in analyzing a rare component of a complex microbial community - and there is plenty more to explore. Quake's team has already begun using the chip to isolate, identify, and sequence communities of microbes that reside in termite hindguts, and his lab at Stanford is custom building chips for other scientists interested in pursuing any culture-resistant microbe or discovering the dark matter of a specific environment.