A new method for assessing the microbiome of the human gut

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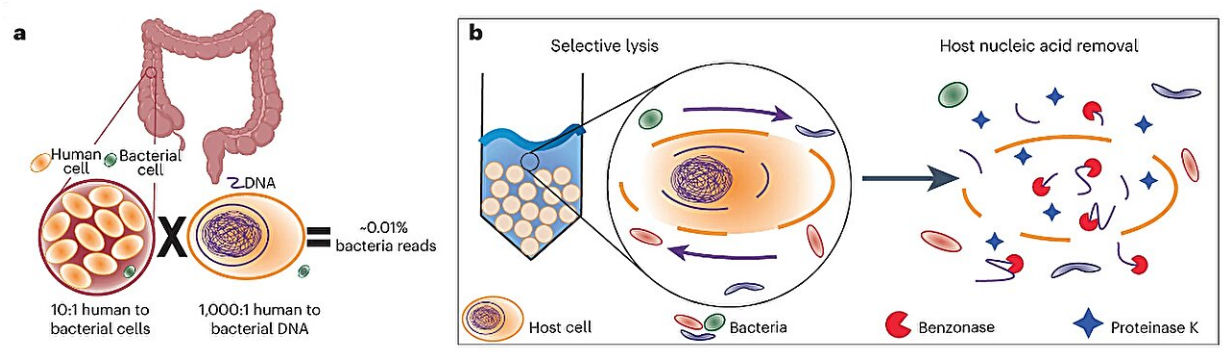

a, Estimated percentage of bacterial reads obtained when human intestinal biopsies are sequenced without processing. b, Schematic demonstrating the two-step selective-lysis and nucleic-acid removal techniques used in MEM. Credit: *Nature Methods* (2023). DOI: 10.1038/s41592-023-02025-4

The gut microbiome—the population and variety of bacteria within the intestine—is thought to influence a number of behavioral and disease traits in humans. Most obviously, it affects intestinal health. Cancer, inflammatory bowel disease, and celiac disease, for example, are all affected by the gut microbiome.

But recent research at Caltech and other research centers has identified connections between the gut microbiome and diseases such as Parkinson's disease and multiple sclerosis as well as links between the

gut microbiome and the presence of autistic behaviors, anxious behaviors, and a propensity to binge-eat sweets. Most of this work has been done in the laboratory of Sarkis Mazmanian, Caltech's Luis B. and Nelly Soux Professor of Microbiology, who works mainly on mouse models.

Looking directly at the human gut and the bacteria that make this space their home is often performed with sequencing—a process that analyzes the DNA sequences that make up each organism. However, this process is difficult in the intestine largely because the amount of microbial DNA in the gut is miniscule in comparison to the amount of host DNA. In intestinal tissue, roughly 99.99% of the DNA present is from the host organism; only 0.01% is microbial DNA.

However powerful the effects of these microbes, it is hard to understand their role without knowing their composition. Microbiome studies often rely on studies of feces and saliva, but these are quite different from the ecosystem of the gut itself.

Now, Natalie Wu-Woods, a graduate student in bioengineering at Caltech in the laboratory of Rustem Ismagilov, the Ethel Wilson Bowles and Robert Bowles Professor of Chemistry and Chemical Engineering, Merkin Institute Professor, and director of the Jacobs Institute for Molecular Engineering for Medicine, has pioneered a simple and effective method to isolate microbial cells from host cells.

This microbial enrichment method (MEM) is "based on the size difference between the host cells and the bacterial cells," Wu-Woods says. "Mammalian cells are about tenfold larger than bacterial cells. We can remove, or at least greatly reduce, the number of mammalian cells through a technique called 'bead beating.' Basically, you take millimeter-sized beads, put them in with the sample you want to study, and then put them on a very fast shaker. The beads break up—or lyse—the larger

mammalian cells, leaving the bacterial cells intact." Then, nucleases—enzymes that degrade free DNA—and protein-destroying proteases are added to remove the remaining host cells and degrade their remains.

In the new study titled "Microbial-enrichment method enables high-throughput metagenomic characterization from host-rich samples" published in the journal *Nature Methods*, Wu-Woods and her colleagues compared MEM to other techniques currently being used to selectively lyse host cells from gut microbiome samples.

She examined four different types of samples—fecal samples from mice; saliva from humans; mucosal scrapings from mouse intestines; and rat colonic sections (similar to human intestinal biopsies)—and found that MEM generally performed as well or better than other techniques on fecal samples, saliva, and mucosal scrapings, and dramatically better on hard-tissue samples, depleting host cells a thousandfold while preserving the microbiome.

Wu-Woods is motivated to discover better methods for separating the gut microbiome from the human host cells in part because researchers can then more easily perform a technique known as shotgun sequencing (in which the microbe's DNA is broken into fragments prior to sequencing) to examine the genomes of gut microbes. Currently, this characterization is done with a method known as 16S rRNA gene sequencing, which provides less information than shotgun sequencing.

"From 16S sequencing you get taxonomic names of the relevant bacteria, but you don't necessarily learn how these bacteria function," Wu-Woods says. "I'm hoping the use of MEM followed by shotgun sequencing will help clarify some of the microbiome health links, as it will allow us to study the mechanism that drives these correlations."

After the initial studies in animal models, Wu-Woods performed MEMs on intestinal biopsies taken from human volunteers who were being screened for colon cancer. Again, MEM succeeded in removing almost all host cells. Wu-Woods then analyzed samples with shotgun sequencing and, for the first time, reconstructed dozens of genomes from low-abundance bacteria originating in human tissues.

"With MEM, we were able to get pretty good sequencing data and actually recreate genomes. This means we can get the entire bacterial genome from an intestinal biopsy without needing to culture cells," says Wu-Woods. She found a treasure trove of bacterial information in each MEM-prepared biopsy, identifying hundreds of bacterial species and genetic pathways and hundreds of thousands of bacterial genes. Incredibly, most of these bacterial genes were unique to each individual, emphasizing that while our own genes are virtually identical among us, the genes of bacteria living in our tissues are incredibly diverse.

More information: Natalie J. Wu-Woods et al, Microbial-enrichment method enables high-throughput metagenomic characterization from host-rich samples, *Nature Methods* (2023). DOI: 10.1038/s41592-023-02025-4

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