UNDER THE LENS

Microbiome cartography

Mathew Stracy

This month's Under the Lens discusses new strategies to image the spatial geography of microbial communities.

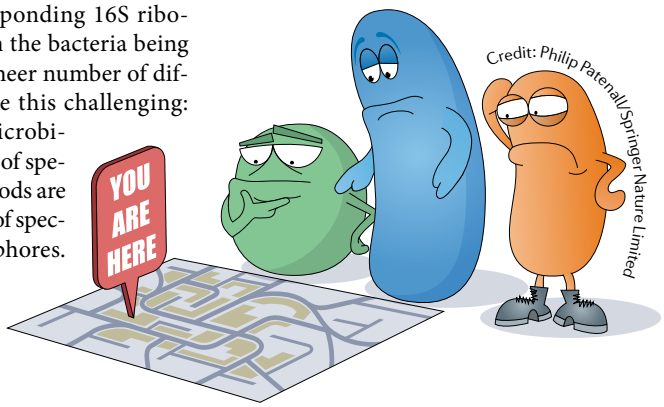
Although metagenomic and transcriptomic techniques have revolutionized microbiome research, these -omic methods only reveal half the picture as they provide no information on the spatial organization or biogeographical heterogeneity of microbial communities. Because different species thrive in different ecological niches, characterizing the biogeography of microbiomes is hugely important to understand how the communities function. Environmental conditions can change over microscopic scales, leading to heterogeneity in gene expression and phenotypes. Furthermore, the spatial proximity between different species affects how they interact, which can ultimately determine the stability of the entire community[1]. The proximity of microorganisms to host cells is also crucially important and can be a key indicator of disease[2].

To explore the uncharted territory of microbial communities at the microscale, researchers are pushing the boundaries of a well-established method: fluorescence in situ hybridization (FISH). FISH can be used to identify species by designing fluorescently labelled nucleic acid probes that hybridize to the corresponding 16S ribosomal RNA sequence in the bacteria being studied. However, the sheer number of different species can make this challenging: the mammalian gut microbiome is host to hundreds of species, whereas FISH methods are limited to just a handful of spectrally resolvable fluorophores. Multiplexed methods can overcome this limitation by labelling different species with a unique combination of fluorophores and using spectral imaging to identify the unique emission 'fingerprint'[3,4].

Using this approach, Shi et al. used ten different fluorophores to distinguish between more than 1,000 strains[4]. The method used a two-step approach to the probe design, which increases versatility and cuts costs. First, species-specific, nonfluorescent, primary probes are hybridized. These probes have overhangs that carry the sequences for a specific combination of fluorescent readout probes. Next, the readout probes are hybridized to the primary probes to create the unique combination of fluorophores for that species. To help decode the confocal spectral image stacks into species information, the team trained a machine-learning classifier.

Shi and co-workers applied the method to image the mouse gut microbiome, allowing quantification not just of species abundance but also of spatial clustering and the number of inter-species physical contacts. By comparing mice with and without antibiotic treatment they found that antibiotics disrupted the spatial associations between several genera, including some linked to altered inflammatory responses in the host.

FISH can also be used to spatially map gene expression by hybridizing to mRNA molecules. Dar et al. took this approach to map the changes in gene expression within biofilms[5]. The team also used a two-step labelling approach, with nonfluorescent primary probes followed by fluorescent readout probes. Instead of a combinatorial approach they used a sequential method: the short, readout probes can be stripped and removed from the sample without affecting the primary probes. A new set of readout probes are then hybridized to read the expression of different mRNAs, allowing hundreds of genes to be measured sequentially in the same sample. Using this method, they found areas of differential virulence factor biosynthesis within *Pseudomonas aeruginosa* biofilms.



Using these imaging techniques to complement -omic approaches will help fill in the gaps in our understanding of how microbial communities are organized. Applying these methods to better understand the architecture of the human microbiota has the potential to impact many aspects of human health, from intestinal diseases to how antimicrobial resistance genes spread.

Mathew Stracy
Sir William Dunn School of Pathology, University of Oxford, Oxford, UK.
e-mail: mathew.stracy@path.ox.ac.uk

https://doi.org/10.1038/s41579-022-00732-x

1. Coyte, K. Z. & Rakoff-Nahoum, S. Understanding competition and cooperation within the mammalian gut microbiome. *Curr. Biol.* **29**, R538–R544 (2019).
2. Nguyen, J., Pepin, D. M. & Tropini, C. Cause or effect? The spatial organization of pathogens and the gut microbiota in disease. *Microbes Infect.* **23**, 104815 (2021).
3. Mark Welch, J. L. et al. Spatial organization of a model 15-member human gut microbiota established in gnotobiotic mice. *Proc. Natl Acad. Sci. USA* **114**, E9105–E9114 (2017).
4. Shi, H. et al. Highly multiplexed spatial mapping of microbial communities. *Nature* **588**, 676–681 (2020).
5. Dar, D. et al. Spatial transcriptomics of planktonic and sessile bacterial populations at single-cell resolution. *Science* **373**, eabi4882 (2021).

Competing interests
The author declares no competing interests.