Microbiomes

https://doi.org/10.1038/s41587-023-01996-1

Spatial methods for microbiome–host interactions

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Spatial transcriptomics offers a glimpse of how microbiomes interact with their hosts.

Our understanding of the ecology of microbiomes has long been constrained by a lack of tools to study microbe–microbe and microbe–host spatial interactions. Two studies in *Nature Biotechnology*, from the groups of Stefania Giacomello[1] and Sanja Vickovic[2], now offer solutions to this problem. Both methods enable exploration of the 'interactome' of host, bacteria and fungi in situ (Fig. 1). By spatially mapping the transcriptomic activity of the host and the microbiome, they reveal at a local scale the intricate dance between host and microbiome. If remaining technical limitations can be overcome, these tools have the potential to offer a new dimension to microbiome research.

Microbiome studies today depend heavily on DNA or RNA sequencing of microbial genetic material obtained from bulk environmental samples. These sequencing-based approaches erase all microbial spatial context information and do not capture bidirectional interactions between microbes and their host. The work of Giacomello and colleagues, and of Vickovic and colleagues, builds on recent methods for spatial transcriptomics that use RNA sequencing with arrayed reverse transcription oligo-dT primers tagged with position-specific barcodes[3–6]. The barcodes and complementary DNA copies of the RNA transcripts are recorded together by sequencing, which enables researchers to create a map of RNA transcript abundance as a function of location in the tissue. However, these methods are largely limited to capturing poly-A-tailed host messenger RNAs and are insensitive to microbial RNAs, which lack a poly-A-tail[7].

The new methods modify a subset of the reverse transcription primers of the array (Fig. 1). This adaptation allows for co-capture of key bacterial and fungal RNA molecules that are highly species-specific and can aid in phylogenetic placement. As the methods are sequencing-based, they offer high taxonomic resolution; this in turn enables the broad detection and mapping of bacterial and fungal species.

Giacomello and colleagues modified the transcript capture array to enable co-capture of 16S rRNA of bacteria and ITS (internal transcribed spacer) and 18S rRNA of fungi (Fig. 1). They used this method to explore the spatial patterning of microbial colonization on *Arabidopsis thaliana* leaves, charting bacterial and fungal reads in leaves without sacrificing host information. The data suggest that spatially restrained microniches of bacteria and fungi correlate with the regional phenotype of the host. Focusing on these 'microbial hotspots' across different leaf sections, the researchers observe a substantial overlap in host gene expression associated with bacterial and fungal RNA presence. This suggests a generalized host response to the presence of microbes, with particular emphasis on chloroplast-related functions that are highly associated with microbial interactions.

Vickovic and colleagues modified a subset of the reverse transcription primers of the capture arrays to include sequences that target the 16S rRNA of bacteria. The resulting method was applied to investigate the effect of microbes on the host — in this case, the mouse gut (Fig. 1).

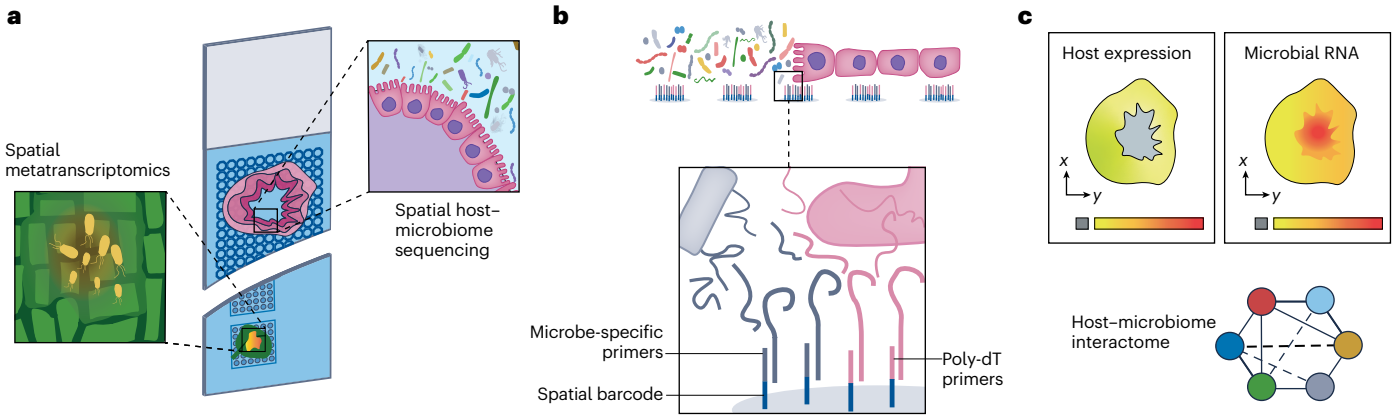

Fig. 1 | Methods to explore the host-microbiome interactome. a, Two related methods use modified spatial transcriptomics to profile the host–microbiome interactome. Bottom left, schematic of spatial metatranscriptomics, a method to study interactions among host, bacteria and fungi on *A. thaliana* leaves. Top right, illustration of the spatial host–microbiome sequencing assay, developed to study the effect of microbes on local host phenotypes in the mouse gut. **b**, Host mRNA and microbial RNA are released from permeabilized tissue and are captured simultaneously by barcoded reverse-transcription primers. **c**, After DNA sequencing, the spatial barcode and the captured RNA information are used to reconstruct spatial maps of host gene expression and microbial abundance. Joint analysis of the two modalities enables the study of the host–microbiome interactome.

By combining histology with spatial transcriptomics and microbial profiling, they revealed the association of specific taxa with anatomical regions in the gut and with expression programs of the host. Key findings from this study include the identification of distinct subsets of goblet cells and colonocytes that, in the presence of microbes, create adhesive layers by expressing MUC2 and CEACAM1 and facilitate host–microbial communication.

Although these two methods are promising, there exist clear opportunities for further innovation. One concern is the limited accessibility of bacterial and fungal RNA to the transcript capture arrays, which reduces sensitivity. Establishing protocols to achieve adequate lysis and RNA release for host cells, Gram-positive and Gram-negative bacteria, and fungi simultaneously is a non-trivial task. Variations in accessibility could skew the interpretation of microbial abundance measurements. Furthermore, ensuring sample integrity and maintaining reliable contact between the tissue and the transcript capture array may be challenging, particularly for samples with fecal matter. Another limitation is the low spatial resolution achieved by these methods. As the spatial resolution is determined by the feature sizes of the transcript capture array (100 µm in the study by Vickovic and colleagues, and 55 µm in the study by Giacomello and colleagues), this could be improved by adopting spatial RNA-sequencing platforms such as Slide-seq and Stereo-seq, which have feature sizes of 10 µm and 220 nm, respectively[4,8]. Additionally, analysis of microbial transcripts can be confounded by environmental contamination and errors in microbial reference databases. Finally, the invasive nature of tissue sampling limits the applicability of these techniques for in vivo studies.

Further advances may include the integration of complementary technologies. For example, fluorescence in situ hybridization techniques can provide a unique perspective on the spatial organization of the microbiome[9–11] and combining them with transcriptomics could lead to a more detailed spatial picture of complex host–microbiome relationships. Computational techniques to integrate and visualize host transcriptome and microbiome data will be essential to fully harness the potential of these new approaches. Lastly, integration with emerging technologies, such as non-invasive gut sampling devices[12], might provide further avenues to overcome limitations related to tissue sampling.

Spatially resolved metagenomics offers a new dimension in microbiome research, enabling exploration of the multifaceted interactions between microbes and their hosts. After further refinement of the methods, we anticipate a wealth of applications in research areas such as host–pathogen interactions, the tumor-associated microbiome, pathogen–microbiome interactions on plant roots and leaves, and microbiome–immune system crosstalk in human disease. Thus, the implications extend widely into diagnostics, agriculture, drug development and other fields, promising to shed light on the intricate complexities of life on a microscopic scale.

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Published online: 20 November 2023

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Competing interests

I.D.V. is a co-founder of Kanvas Biosciences. I.N. declares no competing interests.