Microbiology

Bacterial species singled out from a diverse crowd

Jen Nguyen & Carolina Tropini

Microscopy methods that reveal the spatial patterns of individual types of microbe are limited by the number of different species that can be monitored together. A new technique now provides progress on this front. **See p.676**

Understanding an ecosystem requires maps of where the organisms present live and act. For example, sea otters reside on the surface of kelp beds and feed on sea urchins that consume kelp from the ocean floor. An organism's location in an ecosystem reflects its physiology and function. This is particularly true for microbes, which live in and respond to highly dynamic and diverse habitats. Micrometre-scale gradients of nutrients or pH, for instance, are a typical feature of microbial habitats that drive bacterial spatial organization and behaviour[1,2]. Yet, despite the incredible range of functions that different bacteria perform independently and in association with other living systems[3,4], most microbes are similar in shape and indistinguishable under the microscope. On page 676, Shi *et al.*[5] present a method to tackle the major challenge of differentiating between the hundreds to thousands of bacterial species found in microbes' natural habitats.

A key tool used in biogeographical studies to assess the spatial location of components of interest is called fluorescence *in situ* hybridization (FISH). This technique relies on the use of fluorescently labelled nucleic-acid sequences called probes to locate matching specific sequences of DNA or RNA in a sample that has been immobilized by a process termed fixation. When applied to samples containing bacteria, a DNA probe can be used to identify a target species in the context of its native environment, and a fluorescent molecule (a fluorophore) attached to the probe enables the location of the species to be observed under the microscope[6].

Designing the DNA component of FISH probes is now relatively easy[7]. However, the maximum number of bacterial species identifiable by FISH within a single sample has been constrained by the limited number of fluorophores available for simultaneous visualization. Methods that exceed the limits of traditional fluorescence microscopy, using spectral imaging and combinations of fluorophores, can distinguish between 15 and 120 different types of microbe captured in the same image[8,9]. But a drawback of these techniques is the cost of the large number of fluorescently tagged probes needed.

Shi and colleagues introduce a method that exceeds previous FISH benchmarks by combining a new type of probe design with custom image analysis. Their technique (Fig. 1), named high phylogenetic resolution microbiome mapping by fluorescence *in situ* hybridization (HiPR-FISH), builds on a combinatorial strategy in which bacteria are targeted and labelled in two steps[10].

First, a DNA probe, described as an encoding probe, is designed to match a species-specific sequence (16S ribosomal RNA) for the targeted bacteria. This encoding probe is flanked on either side by integral parts of the probe described as readout sequences. Each of these two readout sequences in an encoding probe can be one of ten possible readout sequences. Then, each readout sequence is targeted by another DNA probe fused to a fluorophore that is specific for the particular readout sequence.

Bacterial cells contain hundreds of copies of 16S rRNA, and so each bacterial species can be targeted by an array of encoding probes: each targeting the same RNA sequence, yet flanked by different pairs of readout sequences, enabling a variety of readout sequences to be associated with a particular species. Choosing the readout sequences for each targeted bacterial species allows the assignment of a unique combination of readout sequences (and therefore fluorophores) to correspond to each species. Depending on the encoding probes used, each bacterial species can bind up to ten of the possible fluorophores. Thus, this system can generate 1,023 unique visual

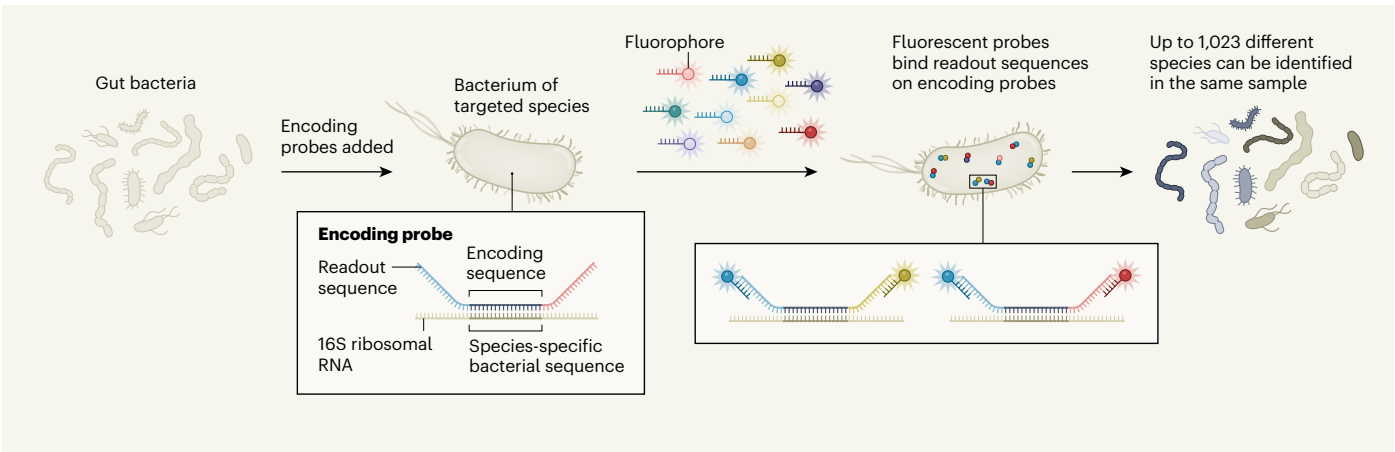

Figure 1 | A method to identify more than 1,000 bacterial species at high spatial resolution. Shi *et al.*[5] present their technique called high phylogenetic resolution microbiome mapping by fluorescence *in situ* hybridization (HiPR-FISH). In this approach, DNA sequences called encoding probes are designed to match a species-specific sequence of RNA (16S ribosomal RNA is the sequence targeted for every species). Each encoding probe is flanked by two sequences called readout sequences, which are each chosen from ten possible readout sequences. A variety of encoding probes that target the same species-specific sequence are designed to generate a unique combination of readout sequences that corresponds to a particular species. Ten types of probe attached to a fluorescent molecule called a fluorophore are added, and each different probe binds the one readout sequence that it matches. The sample is imaged to record which of the fluorophores (up to ten in total) are bound to RNA in each cell. This information enables the genetic identification of individual bacteria.

'barcodes' for the identification of individual bacterial species. Bacterial identities are simultaneously assigned through monitoring by spectral imaging and classification of microbes in the images using a machine-learning algorithm. HiPR-FISH bypasses the previous financial constraints by simplifying the encoding sequence to cheaply synthesized DNA sequences and requiring only ten different types of fluorophore.

Using an automated program to delineate a multitude of single cells in a dense crowd, Shi and colleagues used HiPR-FISH to locate and determine the species identity of individual bacteria in samples from the mouse gut and in samples of oral microbes in human plaque. These different microbial ecosystems are both examples of bacterial communities that can contain hundreds of distinct species.

This demonstration of a previously impossible level of analysis of complex communities using single-cell level mapping, enables the quantitative study of bacterial spatial organization, such as the determination of the distance between specific microbial species residing in a host. Such high-resolution data are important to answer key questions concerning the behaviour of microbial communities, such as who interacts with whom, and where those interactions take place. Interactions are theoretically possible between microbes in close spatial proximity, so HiPR-FISH opens a new era in the study of microbial ecology by enabling micrometre-scale mapping of the spatial distance between hundreds of microbial species in complex communities.

Shi *et al.* assessed the distance between different bacterial species normally resident in the mouse gut and measured how these distances changed after antibiotic treatment. Such therapy is known to alter the assortment and abundance of bacterial species in the gut[11]. The largest distance changes due to antibiotic treatment observed by Shi and colleagues were between *Oscillibacter* and *Veillonella*, which are microbes that are both individually associated with health benefits in the human gut[12,13]. Whether and how these bacteria interact functionally remains to be uncovered. However, the fourfold increase in spatial distance between these bacteria after antibiotic use raises the possibility that the antibiotic treatment might disrupt an interaction that aids the host. Identifying such interactions and deciphering the underlying mechanisms will boost our understanding of how microbial communities respond to, and recover from, environmental perturbation.

By shedding light on microbial biogeography, this work charts new paths for exploring microbial interactions in complex ecosystems. Exciting next steps to anticipate include the elucidation of mechanisms by which environmental disturbances alter bacterial spatial organization, and how altered organization affects community function. For example, how does antibiotic exposure result in an increase in distance between *Oscillibacter* and *Veillonella*? Is the spatial proximity between specific bacteria important for community recovery after disturbances such as antibiotic exposure?

Finally, expanding on this technology to access the spatial organization of transcriptional responses would enable maps to be generated that reveal the spatial gradients of bacterial species and their functions. These future applications will truly revolutionize our understanding of complex microbial communities and the spatial diversity that is so fundamental for life.

Jen Nguyen and **Carolina Tropini** are at the School of Biomedical Engineering and the Department of Microbiology and Immunology, University of British Columbia, Vancouver V6T-1Z3, Canada. **C.T.** is also at the Humans and the Microbiome Program, Canadian Institute for Advanced Research, Toronto, Canada.
e-mail: carolina.tropini@ubc.ca

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Biogeochemistry

Making the most of wetland restorations

Jacques C. Finlay

Wetlands remove nitrate pollution from water effectively. An analysis shows that this effect is constrained in the United States by the distribution of wetlands, and could be increased by targeting wetland restoration to nitrate sources. **See p.625**

Human activities have increased the amounts of reactive nitrogen compounds — forms of nitrogen that can be used by organisms for growth — in the environment. The resulting overabundance of reactive nitrogen has far-reaching consequences for ecosystems, climate, and human health and well-being[1]. Fertilizers are the largest global source of anthropogenic nitrogen inputs[2], and so intensive efforts have been made to reduce nitrogen transport from agricultural land to ground and surface waters, but with mixed results. On page 625, Cheng *et al.*[3] report that sources of river nitrogen pollution in the United States are often spatially separated from existing wetlands (Fig. 1), which can remove nitrate from water, and show that wetland restoration targeted to nitrate sources would yield substantial benefits for downstream water quality.

The beneficial effects of wetlands on water quality are well documented, and wetlands are widely used both in urban and rural settings to remove pollution arising from human activities[4]. The biogeochemical conditions in wetlands particularly favour the removal of nitrate, which is often the dominant form of nitrogen pollution in water. However, the global area of wetlands has reduced drastically over the past two centuries[5,6], and losses continue despite greater protections being established. The need for wetland restoration is clear, but it is difficult to calculate the potential contributions that restorations could make to nitrate removal for large water catchment areas by scaling up the effects of individual wetlands. This is because water-quality outcomes are highly sensitive to the geographical distribution of wetlands relative to that of nitrogen sources[7–9].

Cheng *et al.* tackle this problem by combining an inventory of US wetland distribution with models of nitrogen transport. Their analysis affirms — with much greater precision than was possible in past studies — that remnant and restored wetlands in agricultural areas have a disproportionately large role in mitigating