MICROBIOLOGY

Keeping track of gut bacteria

Fluorescent labeling of bacteria enables tracking of their location.

The mammalian gut is a long tube with nooks and crannies, each of which is biologically quite different from the others. Microbes, metabolites, and epithelial and immune cells vary in composition and function along the gut, so tracking bacteria's location can provide valuable information. In a recent study, Dennis Kasper and colleagues fluorescently labeled the anaerobe *Bacteroides fragilis* and imaged its passage through the mouse intestine *in viv* and *ex vivo*.

Approaches used previously to track bacteria include fluorescence *in situ* hybridization (FISH) and genetic manipulation to tag microbes with fluorescent proteins. But FISH is limited by the time-consuming nature of a tissue section–based approach. Genetic manipulation of gut bacteria is not trivial, and fluorescent proteins require oxygen, which is in low abundance in the intestine and its inhabitants.

Instead, the Kasper group labels bacteria with a fluorophore using metabolic oligosaccharide engineering, incorporating azide-modified sugars into growing cells. A cyclooctyne fluorophore can then be added as a tag via bio-orthogonal click chemistry. The researchers found that this approach labels polysaccharide A, known to mediate *B. fragilis*'s immunomodulatory functions and the major polysaccharide on the surface of the Gram-negative bacteria.

The approach makes it possible to image *B. fragilis* in a mouse model of peritonitis, where it disseminates to the spleen, and the cells that phagocytose the bacteria *in vivo* can be characterized using flow cytometry. The researchers quantified total *B. fragilis* in the murine intestinal tract by imaging a whole live animal over time, and they obtained specific locations for the bacteria by imaging intestinal organs *ex vivo*.

Kasper and colleagues labeled nine other bacteria and showed that mice can be inoculated orally with a mix of three species tagged with separate fluorophores to identify compositional changes in different regions of the intestinal tract using flow cytometry.

The approach does not easily distinguish between the fluorescent signal from cells and that from labeled carbohydrate that was shed. Also, chemical labeling of live bacteria results in progressive loss of the signal as they proliferate. Still, the method is likely to find multiple applications in studies of the microbiota in a host

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RESEARCH PAPERS

Geva-Zatorsky, N. *et al. In vivo* imaging and tracking of host-microbiota interactions via metabolic labeling of gut anaerobic bacteria. *Nat. Med.* **21**, 1091–1100 (2015).