Three-dimensional reconstruction of B. theta’s localization in the gut

Background reading:


Introduction:

The human intestinal microbiota is home to hundreds of bacterial species that comprise a dense microbial ecosystem. As in other microbial ecosystems, previous studies have suggested that the bacteria within the intestine are not randomly distributed. There are numerous factors that may organize where different bacteria localize, including: length along the intestine (i.e., ileum, cecum, proximal, transverse, and distal colon), proximity to intestinal epithelium (i.e., adherent to epithelium, loose mucus-associated, or in digesta), and other discrete locations like crypts and spaces between transverse folds in the colon (Figure 1).

Until somewhat recently, sampling and fixation methods have limited the ability to localize bacteria in relatively intact intestines. The anaerobic environment has also made direct imaging of fluorescent bacteria difficult. Fluorescence in situ hybridization (FISH) and immunohistochemistry have been used to determine with somewhat low resolution the localization of divisions of bacteria in complex mouse microbiotas.

This project aims to attain high-resolution 3D images of a single bacterial species in the mouse intestine. To do this, we will be using a gnotobiotic mouse model and a model gut symbiont, *Bacteroides*.
B. theta is a common component of the human microbiota, and a representative of the dominant genus of Westerners (Eckburg et al, Science, 2005). It possesses an expansive saccharolytic capacity, able to metabolize a broad range of dietary polysaccharides and host mucosal glycans. Previous studies using FISH have suggested that members of the genus Bacteroides localize primarily to the digesta, distant from both mucus and transverse folds. In this project, we will use our model system to get a better picture of where B. theta localizes in a mono-, and possibly bi-, colonized gut, and how this may be affected by diet changes.

Objective:
Use serial sections of monocolonized ileum and colon to generate 3D reconstructions of B. theta in the gut. Use MATLAB image analysis tools to quantify bacterial density and location in the gut relative to each other and the host. Design a graphical user interface (GUI) to facilitate image analysis. Given sufficient progress, additional images generated from dietary changes and/or inclusion of the human gut pathogen C. difficile may be analyzed.

Primary questions:
1. In monocolonized mice, how closely do B. theta associate with the colonic epithelium? How does this compare with existing data in a complex microbiota?
2. What is the density of B. theta cells in the ileum and the colon?
3. What is the average distance between cells? Are cells uniformly distributed?

Secondary questions if time permits:
1. Does diet change (i.e., reliance on host mucins vs. dietary carbon sources) change localization?
2. Does syntrophy between two species (B. theta and C. difficile) necessitate a close association and/or drive a change in the localization of B. theta?