

**Title:**

Phenotypic Specialization of *Y. pseudotuberculosis* within Microcolonies

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**Abstract:**

Many enteric bacterial pathogens can spread from the intestines to deep tissue sites, where infections become difficult to treat with antibiotics. *Yersinia pseudotuberculosis* is an example of an enteric pathogen that spreads to deep tissue sites, such as the spleen, where it replicates to high numbers despite the recruitment of host phagocytes. Within the spleen, bacteria replicate extracellularly to form clusters, called microcolonies. Bacteria around the periphery of microcolonies come into direct contact with neutrophils, and must prevent phagocytosis and extracellular killing, however interior bacteria only contact other bacteria. We hypothesize this drives phenotypic specialization of individual bacteria, based on their spatial location relative to host cells. These studies focused on the bacterial nitrogen stress response, and the production of host nitric oxide. We utilized bacterial fluorescent reporter constructs to visualize expression of the nitric oxide detoxifying gene, *hmp*, at the single cell level. Fluorescence microscopy of infected tissues was used to visualize reporter expression. Bacteria located around the periphery of microcolonies responded to nitrogen stress by expressing high levels of *hmp*. In contrast, *hmp* expression was not detected from interior bacteria, indicating peripheral bacteria may effectively eliminate the nitric oxide gradient in tissue. *hmp* expression by peripheral bacteria promoted bacterial survival within tissue sites and impacted virulence, based on experiments with a  $\Delta hmp$  strain. Immunofluorescence microscopy was used to visualize the location of nitric oxide-producing host cells relative to microcolonies. Neutrophils in contact with the microcolony do not express nitric oxide, instead this is produced from host cells distant from the microcolony. Contact between host cells and peripheral bacteria also led to heightened virulence factor expression (*YopE*), relative to interior bacteria. *Yop* expression is required for virulence in the spleen, and high expression of *Yop* genes may also define peripheral bacteria. These studies identify the existence of multiple subpopulations of bacteria present within a single microcolony, and also suggest cooperative behavior must be established to support bacterial replication within tissue sites.