

## **Hochstedler - Microbiology and Immunology**

**Abstract:** The natural history of recurrent and chronic urinary tract infections is currently not defined; however, the underlying cause of these conditions is hypothesized to be the establishment of intracellular bacterial communities by uropathogenic organisms. Recently, the urinary microbiome field has identified an increased prevalence of *Enterococcus faecalis* in recurrent and chronic urinary tract infection populations. In 2013, Dr. James Malone-Lee's group analyzed shed bladder (urothelial) cells from urine of chronic urinary tract infection patients and found evidence of intracellular *E. faecalis*. After isolation, further analysis using a novel bladder organoid model showed these *E. faecalis* strains exhibited increased invasion of urothelial cells compared to *Escherichia coli* strains isolated from the same population. Understanding the mechanisms responsible for this invasive *E. faecalis* phenotype will lead to increased knowledge of enterococcal pathogenesis and improvements in patient care. Additionally, extending the applications of this bladder organoid model, which very closely mimics the human bladder environment, will significantly advance the urinary microbiome field.

**Undergraduate work:** The bladder organoid model uses a human urothelial cell line (HBLAK) to create microscopic, multi-layered, 3D bladder structures. When treated appropriately, the HBLAK cell line differentiates to create an inner layer of umbrella cells, intermediate layers, and an undifferentiated basal cell monolayer. The resulting bladder organoids can then be infected with uropathogens, such as *E. faecalis*. The invasion and attachment of uropathogenic organisms and urothelial cells can be observed by 3D confocal microscopy and quantified by gentamicin protection assays. The above model and methodology is already established in the Wolfe lab. While the summer project will include data collection for *E. faecalis* mutants pertinent to my dissertation research, the main training focus for the summer undergraduate student will encompass basic tissue culture and qualitative analysis techniques by working alongside me to create and maintain the bladder organoids. Additional training will include exposure to basic microbiological techniques for qualitative and quantitative analysis of organoid experiments. This will include observing and helping with fluorescent staining, infecting, and analyzing images of bladder organoids. Additionally, the undergraduate student will be able to observe and participate in identification and quantification of adherent and invasive bacteria via image analysis software and gentamicin protection assays.