M & I
Microbiology
and Immunology
University of North Carolina at Chapel Hill

DISSERTATION SEMINAR

Robert McKee

"The c-di-GMP regulatory network in *Clostridium difficile* and its role in modulating surface adherence and persistence in the mammalian gut."

Friday, April 20, 2018
2:00 p.m.
1131 Bioinformatics

Dissertation Advisor: Dr. Rita Tamayo

Presented in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

ABSTRACT

Robert McKee: "The c-di-GMP regulatory network in *Clostridium difficile* and its role in modulating surface adherence and persistence in the mammalian gut (Under the direction of Dr. Rita Tamayo)

Clostridioides difficile (*Clostridium difficile*) is a spore-forming bacterial pathogen responsible for hundreds of thousands of infections each year in the United States. *C. difficile* outbreaks are common in hospitals because *C. difficile* spores can persist for months on surfaces and are resistant to many disinfectants. Despite the significant disease burden that *C. difficile* represents, we know surprisingly little about the factors necessary for *C. difficile* to colonize and persist in the mammalian intestine. Previous work demonstrated that the signaling molecule cyclic diguanylate (c-di-GMP) regulates a variety of processes in *C. difficile* including production of the toxins that are required for disease symptoms. Using monolayers of human intestinal epithelial cells, we demonstrate that c-di-GMP promotes attachment of *C. difficile* to intestinal epithelial cells. We also demonstrate that regulation of type IV pili (TFP) by c-di-GMP promotes prolonged adherence of *C. difficile* to epithelial cells *in vitro*. *C. difficile* mutants lacking TFP were cleared more quickly than the parental strain during single strain mouse infections and were outcompeted by the parental strain during *in vivo* competition experiments in mice. Thus, our data provides evidence that TFP promote persistence of *C. difficile* in the intestine. To determine what other genes c-di-GMP regulates in *C. difficile*, we performed RNA-sequencing comparing the transcriptome of *C. difficile* with elevated c-di-GMP to that of *C. difficile* with basal levels of c-di-GMP. We demonstrate that c-di-GMP regulates the expression of 166 genes greatly expanding the known members of the c-di-GMP regulon. We demonstrate that c-di-GMP regulation of several transcripts in *C. difficile* is dependent on c-di-GMP sensing riboswitches present in the 5' untranslated regions of these transcripts. Our results also show that c-di-GMP regulates a number of cell envelope proteins in addition to TFP and flagella. These data suggest a broader role for c-di-GMP in remodeling the *C. difficile* cell surface.