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

DISSERTATION SEMINAR

Brandon R. Anjuwon-Foster

"Interstate 5': The Rho(d) to Termination."

Friday, April 27, 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

Brandon R. Anjuwon-Foster: Phase Variation of *Clostridium difficile* Virulence Factors
(Under the direction of Dr. Rita Tamayo)

Clostridium difficile is a Gram-positive spore-forming anaerobe and the leading cause of antibiotic-associated diarrheal disease in the United States. *C. difficile* produces two toxins, TcdA and TcdB, that are necessary for diarrheal disease symptoms. Colonization of the intestine is a necessary prerequisite to diarrheal disease symptoms. *C. difficile* produces flagella that aid not only in bacterial motility, but adherence to intestinal tissue. SigD, a flagellar alternative sigma factor in the early stage flagellar (*flgB*) operon, indirectly activates expression of the *tcdA* and *tcdB* genes. Both flagella and toxins are *C. difficile* virulence factors that synergistically promote diarrheal disease symptoms, pathology, and inflammation. Therefore, factors that regulate expression of the *flgB* operon affect not only motility, but toxin production and the virulence of *C. difficile*. The main objective of the research described in this dissertation was to identify and characterize a genetic mechanism controlling co-regulated flagellar and toxin gene expression in *C. difficile*. In Chapter 2, we identified a “flagellar switch” located upstream of the *flgB* operon, that mediates the phase variable production of flagella and toxins in *C. difficile*. Bacteria with the sequence in one orientation produced flagella, were motile and secreted the toxins ("*flg* ON"). Bacteria with the sequence in the inverse orientation were aflagellate and showed decreased toxin secretion ("*flg* OFF"). We determined that the tyrosine recombinase RecV is required for inversion of the flagellar switch in both directions. In Chapter 3, we found a single strain family, designated as “ribotype 012”, of *C. difficile* exhibits low frequency inversion of the flagellar switch in laboratory-adapted, environmental, and clinical isolates. In Chapter 4, we demonstrated that Rho factor is required for flagellar phase variation in *C. difficile*. We hypothesize that Rho factor directly terminates transcription in the leader RNA of the *flgB* operon in *flg* OFF bacteria. Future studies will assess the virulence contribution of flagellar and toxin phase variation to host infection dynamics, outcome, and transmission. Phase variable flagellar motility and toxin production suggests that these important virulence factors have both advantageous and detrimental effects during the course of infection.