WEEKLEY1, JACOB PANCAKE1, JENNIFER HICKMAN1, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV, DONALD PRIMERANO2, JAMES DENVIR2, Genomics and Bioinformatics Core Facility, Marshall University, Huntington, WV AND DEANNA M. SCHMITT1. [1]Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV. The role of *pilD* in *Francisella tularensis* susceptibility to resazomycins.

The CDC classifies *Francisella tularensis* as a Category A bioterrorism agent. If used during a terror attack, the inhalation of a single *F. tularensis* bacterium can cause the fatal disease tularemia. Due to the potential release of antibiotic-resistant *F. tularensis* strains, new therapeutics against *F. tularensis* must be developed. Resazomycins are resazurin-based compounds that exhibit antimicrobial activity against *F. tularensis* as well as other Gram-negative bacteria including *Neisseria gonorrhoeae*, the causative agent of gonorrhea. The mode of action of these antibiotics is not understood. To identify potential targets of resazomycins, we selected for mutants of *F. tularensis* that were capable of growing in the presence of 20x the minimal inhibitory concentration of resazurin (Rz). Approximately 50% of these Rz-resistant *F. tularensis* isolates had a mutation in the genes FTL_0959 (*pilD*) and FTL_1306 (*dipA*). In *F. tularensis*, *pilD* encodes for the cytoplasmic membrane peptidase responsible for processing the prepilin subunits in type IV pilin assembly proteins. To investigate the role of *pilD* in Rz susceptibility, we generated a *pilD* disruption mutant in wild-type *F. tularensis* and tested its sensitivity to Rz. In preliminary experiments, growth of the *pilD* disruption mutant is inhibited by Rz, although to a lesser degree than wild-type LVS suggesting *pilD* may play a minor role in resazomycin susceptibility. We are currently working to generate a complementation construct to express wild-type *pilD* in select Rz-resistant *Ft* mutants to further investigate the role of this gene in Rz susceptibility.