KENDALL SOUDER, MICHAEL WHABY, JENNIFER HICKMAN, Department of Biological Sciences, West Liberty University, West Liberty, WV, DONALD PRIMERANO, JAMES DENVIR, Genomics and Bioinformatics Core Facility, Marshall University, Huntington, WV, & DEANNA M. SCHMITT. Department of Biological Sciences, West Liberty University, West Liberty, WV. Contribution of *Francisella tularensis* FTL_1306 (*dipA*) in resazomycin susceptibility.

The CDC classifies *Francisella tularensis* as a Category A bioterrorism agent. Due to the growing global threat of antibiotic resistant bacteria, novel therapeutics against *F. tularensis* must be developed. Resazomycins are resazurin (Rz)-based compounds that exhibit antimicrobial activity against *F. tularensis* and other gram-negative bacteria. The action of resazomycins is not well understood, but potential targets of the antibiotic were identified in a high throughput screen for Rz-resistant isolates. The *dipA* (FTL_1306) gene was identified as mutated in half of the 48 Rz-resistant (RZR) strains sequenced. To further investigate the role of *dipA* in Rz susceptibility, we introduced a wild-type copy of *dipA* into select RZR isolates (RZR1, 5, 43, and 46) that contain *dipA* mutations. The *dipA* gene was amplified by PCR from wild-type *F. tularensis* and cloned into the *F. tularensis* shuttle vector pABST to generate a construct (pABST-*dipA*) in which *dipA* will be constitutively expressed under control of the groEL promoter. The pABST-*dipA* plasmid was mobilized into each of the selected RZR isolates by electroporation. Western blotting indicated that expression of wild-type *dipA* was restored in RZR strains with the introduction of the pABST-*dipA* construct. The MIC of resazurin for the resulting RZR *dipA*-complemented strains was equivalent to that wild-type *F. tularensis* and significantly different from resistant mutants. Further investigation is needed to fully elucidate the contribution of *dipA* to the bactericidal action of resazomycins. *(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)*