

ALEXIS SCOTT, MACKENZIE HALL, EMILY YOUNG, & DEANNA M. SCHMITT. The Hilltop Center for Biomedical Research, West Liberty University, West Liberty, WV 26074. Characterization of Putative Oxidoreductase Involved in Resazomycin Resistance in *Francisella tularensis*.

Antibiotic resistance poses a critical threat to global public health, emphasizing the need to identify and characterize novel bacterial resistance mechanisms. Our laboratory discovered resazurin-based compounds, known as resazomycins, exhibit antimicrobial activity against *Francisella tularensis*, the causative agent of tularemia. Previous studies have shown that overexpression of FTL_0445 in *F. tularensis* confers resistance to resazurin. FTL_0445 is annotated to encode for a NADPH-dependent FMN oxidoreductase that we hypothesize reduces or modifies resazurin, thereby mediating antibiotic resistance in *F. tularensis*. To investigate this hypothesis, the FTL_0445 gene was amplified and cloned into the linearized pET45b (+) expression vector for heterologous expression in *Escherichia coli* using In-Fusion cloning. Following transformation into *E. coli*, colonies were isolated on LB + ampicillin plates, and plasmid DNA was extracted using a mini-prep kit. Restriction digest analysis of purified plasmids revealed DNA fragments of expected sizes: approximately 5300 bp for the pET45b (+) vector and approximately 600 bp for the FTL_0445 insert, confirming successful cloning and proper vector-insert assembly. Currently, efforts are underway to induce protein expression and purify the FTL_0445-encoded protein in *E. coli* for biochemical characterization. Subsequent assays will assess its potential oxidoreductase activity toward resazurin derivatives to further elucidate the gene's role in resazomycin resistance. Understanding the function of FTL_0445 could provide valuable insight into the molecular basis of antibiotic resistance in *F. tularensis* and may guide the future development of novel therapeutic strategies to combat antibiotic-resistant pathogens.