

JANELLE STACK, ADAM JARRETT, and DEANNA M. SCHMITT, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. Investigation of potential dominant negative *dipA* mutants in susceptibility of *Francisella tularensis* to resazomycins.

Multidrug-resistant bacteria pose a significant threat to global public health. According to the CDC, more than 2.8 million antibiotic-resistant infections occur in the United States each year, and more than 35,000 people die as a result. The development of new antibiotics is necessary to combat this public health crisis. Recently, we identified a novel family of compounds, resazomycins, which exhibit antimicrobial activity against select Gram-negative bacteria such as *Francisella tularensis*. The mode of action of resazomycins is not well understood, but through a screen for Rz-resistant (RZR) isolates of *F. tularensis*, one gene, *dipA*, was identified to be mutated in half of the strains that were sequenced. Complementation with wild-type *dipA* did not restore sensitivity to Rz in the RZR isolates, suggesting *dipA* does not play a role in Rz susceptibility or mutant *dipA* may be exhibiting a dominant negative effect. To explore the latter possibility, the *dipA* gene was amplified by PCR from spontaneous Rz mutants RZR43 and RZR46 and cloned into the *F. tularensis* shuttle vector pABST. These recombinant plasmids were then introduced into wild-type LVS. Upon confirmation of the successful introduction of each *dipA* allele into LVS, the resulting strains were then cultivated on chocolate agar containing Rz to determine their susceptibility to resazomycins. Preliminary results suggest that expression of mutant *dipA* in LVS did not confer resistance to Rz indicating *dipA* may not play a role in Rz susceptibility. Further investigation is needed to fully elucidate the contribution of *dipA* to the bactericidal action of resazomycins.

