

*Pseudomonas syringae* is a Gram-negative bacterial plant pathogen that infects economically relevant crops like tomato and soybean in addition to the model host plant *Arabidopsis thaliana*. Similar to various human pathogens, *P. syringae* relies on a type III secretion system (T3SS) to proliferate and cause diseases. The T3SS is essentially a molecular syringe that the bacterium uses to inject an arsenal of proteins called type III effectors (T3Es) directly into host cells. Although we understand that T3Es collectively contribute to disease primarily by suppressing plant innate immunity, the exact molecular mechanisms by which most of these proteins do so to allow disease progression remain elusive. This project is focused on *A. thaliana* histone deacetylase 5 (HDA5), a protein we have previously shown to be involved in deacetylating chromatin along a subset of innate immune genes when *Arabidopsis* is infected with wildtype *P. syringae* DC3000. This deacetylation does not occur with a *hrcC* mutant incapable of translocating T3Es into the plant cell. Our hypothesis is that injection of T3Es into plant cells during a *P. syringae* infection results in HDA5 translocation into the nucleus of *Arabidopsis*. To determine if this localization occurs, we decided to isolate intact nuclei from *Arabidopsis* leave tissue and analyze HDA5 localization and abundance pre- and post- *P. syringae* infections. We have adapted existing nuclei isolation protocols incorporating membrane lysing buffers followed by a series of centrifugation steps and a Percoll density gradient to fractionate plant cell organelles. Once samples were obtained, isolation and purity of nuclei was determined using fluorescence microscopy with the DNA binding dye, DAPI. In addition, we used immunoblotting with anti-histone H3 antibodies and anti-UDP Glucose Pyrophosphorylase to detect nuclear and cytoplasmic fractions respectively in order to determine the purity level of the nuclei. Preliminary data shows clean intact nuclei with minimal cellular debris contamination. This is a promising step toward our goal. We are now investigating the localization of HDA5 upon *P. syringae* infection using transgenic *Arabidopsis* plants expressing HA-tagged HDA5.