Engineering a Bacterial Pathogen with an Enhanced Luminescent Reporter for High-Throughput Resistance Assays

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Bioluminescent strains of Pseudomonas syringae pathovar (pv.) tomato was developed for a high-throughput resistance assay. There has been an effort to generate a pathogen carrying a luminescent reporter. However, existing strains have never been widely used due to their low efficiency. This project was an attempt to engineer a new strain with a brighter reporter to accommodate a high-throughput analysis. To this end, multiple recombinant reporter genes with three NanoLuc Luciferase (NL 1.1, NL 1.2, & NL 1.3) under a constitutive promoter (proD) were constructed. The NanoLuc Luciferase is a recent luminescence reporter enzyme which has been shown to be at least 100 times brighter than conventional counterparts. New recombinant plasmids were introduced to the genome of P. syringae (pst) via a transposon-mediated recombination. These new Pseudomonas strains were tested in vitro as well as in vivo, to assess the luminescence efficiency. 96-well plates were used to compare the luminescence among the new strains. Also, Nicotiana benthamiana was infiltrated with the new reporter strains. In sum, NanoLuc bacterial strains were bright enough to be detectable under an EM-CCD camera and far brighter than the luxCDABE luciferase strains that have been used by plant pathologists for the last several years. In Conclusion, the development of efficient strains presents a great opportunity to further set up a large-scale experiment in which an unprecedented number of plants would be tested with these pathogen strains.

Awards Won:

Air Force Research Laboratory on behalf of the United States Air Force: First Award of $750 in each Intel ISEF Category