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Francisella tularensis FTL_0580 N-Terminal Domain Enhances Fluorescence Protein Expression

Francisella tularensis is a gram-negative, intracellular pathogen capable of establishing a lethal infection at a dose of <10 CFU. Fluorescence microscopy is a vital tool used to study *F. tularensis* host-pathogen interactions. However, *F. tularensis* is only weakly fluorescent when expressing fluorescent proteins such as emGFP and BFP using previously described molecular tools. In contrast, our laboratory previously described robust expression of tdTomato while under the control of the *Francisella* glucose-repressible promoter (FGRp; plasmid construct pTC3D). However, *F. tularensis* bacteria were unable to produce substantial expression of tdTomato or other fluorescent proteins, such as emGFP, while genes encoding these proteins were under the control of the robust *groE* promoter (*groEp*). Therefore, we analyzed the DNA upstream of *tdtomato* in pTC3D and identified an in-frame N-terminal fusion of the first 36 codons of FTL_0580 (580N), followed by code for a three amino linker domain (PAT), and subsequently the start codon of tdTomato potentially producing a stable hybrid protein. The previously identified FGRp alone could not produce robust expression of fluorescent proteins. Moreover, a frame-shift disrupting the coding sequence of 580N resulted in poor fluorescent protein expression. Inclusion of 580N in-frame controlling expression of Emerald GFP, *emGFP*, under the control of FGRp or *groEp*, however, resulted in robust fluorescence. We hypothesize that 580N stabilizes the fluorescent protein to which it is fused within *F. tularensis*. Application of this new biotechnology will expand our ability to investigate *F. tularensis* through the use of fluorescence microscopy. (*Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence* and *NIH Grant 1R15HL147135-01*)