OFFICIAL ABSTRACT and CERTIFICATION

Outer Membrane Vesicle and Tube Formation in Francisella

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Francisella novicida, a laboratory strain of Francisella tularensis, produces outer membrane vesicles and tubes (OMVT) under specific media and growth conditions. OMVT have been associated with several known Francisella virulence factors, providing evidence for their role as a special secretion system during host cell invasion. A major focus of OMVT studies is elucidating the mechanisms underlying their production. To assist this focus, F. novicida was analyzed using cryoelectron tomography to acquire detailed structures of the tubes. It appears that a ' bulb-like ' structure forms within each tube that pushes their formation outwards, suggesting the involvement of many different proteins at this center responsible for regulating OMVT production. Using a genetic screen, several hypo-vesiculating mutants were identified as genes of interest. Two of the strongest hypo-vesiculating genes were FumA (fumarate hydratase, an enzyme involved in carbon metabolism) and FTN_1037 (unknown protein). The purpose of this study was to identify their role in OMVT regulation by analyzing their sub-cellular localization in tube producing bacteria. The goal was to fuse these genes with green fluorescent protein (gfp) using overlap extension PCR, and track their localization, with respect to tube formation, using fluorescence microscopy. The constructs were prepared in the Francisella expression vector – pFNLTP6 and transformed into E. coli and F. novicida. Microscopy was used to verify that the gfp-tagged proteins are functional and to study their localization. This study will help acquire more knowledge about the role of FumA and FTN_1037 which would ultimately help in understanding the mechanisms that regulate OMVT production.

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