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Production of outer membrane vesicles and tube-like structures by *Francisella tularensis*

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Aims: *Francisella tularensis* is a Gram-negative bacterium and the causative agent of tularemia. This organism lacks many of the virulence-associated secretion systems typically found in other intracellular pathogens, such as the type III and type IV pathways. Outer membrane vesicles (OMVs) are constitutively shed from growing Gram-negative bacteria and have been shown to act as secretory vehicles for toxins in other pathogenic bacteria. We examined the production of OMVs by *F. tularensis* to assess their potential as a virulence factor secretion system.
Methods: We isolated OMVs from *F. tularensis* subsp. *novicida* U112 using high-speed centrifugation of filtered culture supernatant fractions. Intact bacterial cells and isolated OMVs were visualized by transmission electron microscopy. Vesicle fractions were further purified via flotation through a density gradient. Purified vesicles were analyzed for protein content using SDS-PAGE and mass spectrometry.
Results: We were able to isolate OMVs from strain U112. Examination of purified vesicles revealed the presence of tube-like structures in addition to spherical OMVs. The tube-like structures were also observed protruding from the surface of intact bacteria. Using mass spectrometry we identified several proteins enriched in the OMV/tubes. Examination of various U112 mutants identified genes associated with type IV pilus production as important for secretion of some vesicle-associated proteins and for OMV/tube production.
Conclusion: *F. tularensis* subsp. *novicida* U112 produces OMVs as well as novel, tube-like structures. The tube-like structures are both secreted into the culture medium as well as present on the bacterial surface. Genes associated with type IV pilus biogenesis appear to play a role in OMV/tube production. The function of the OMVs and tube-like structures is currently unknown, but some of the proteins identified as vesicle-associated were previously shown to be important for *Francisella* virulence.