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Comparative proteome analysis of *Francisella tularensis* LVS and DsbA deletion mutant: identification of potential substrate proteins for the DsbA

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Aims: The conserved hypothetical lipoprotein (FTL_1096) exhibits high homology with proteins of the disulfide oxidoreductase DsbA family. In our previous studies the gene encoding this DsbA homologue was verified to be required for survival and replication in macrophages and also for *in vivo* virulence in the mouse infection model for tularemia. The DsbA proteins are known to be important for toxin secretion or proper folding of outer membrane adhesions in many pathogens. To recognize *F. tularensis* proteins whose folding might be dependent on the DsbA protein activity we investigated the possible membrane accumulation of misfolded proteins in the *dsbA* mutant.
Methods: To examine the potential substrate proteins for the *F. tularensis* DsbA homologue, we performed comparative proteomic analyses of fractions enriched in membrane proteins of LVS and ΔFTL1096 mutants. The fractions enriched in membrane proteins of bacteria grown in defined Chamberlain medium were prepared using carbonate extraction and ultracentrifugation. The obtained protein samples were analyzed using both classical (two-dimensional gel electrophoresis + mass spectrometry identifications) and shotgun (iTRAQ labeling + LC-MS/MS) proteome approaches.
Results: Using the both mentioned proteomic approaches we were able to identify in total seven up-regulated proteins in fractions of the mutant strain. Five proteins do not exhibit any homology to known bacterial proteins and their function has to be further investigated. In addition to the DsbA protein, two other proteins were found down-regulated in *dsbA* mutant.
Conclusions: The proteins differentially expressed in the mutant strain appear to depend on DsbA for localization and function and are candidates for being more directly responsible for its virulence attenuation. Further studies are under way to address the role of these genes in the pathogenesis of tularemia.