

**ABSTRACT****Charles University in Prague****Faculty of Pharmacy in Hradec Králové****Department of Biochemical Sciences****Candidate:**

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Title of Doctoral Thesis:

IDENTIFICATION OF NEW VIRULENCE FACTORS IN INTRACELULLAR PATHOGEN *FRANCISELLA TULARENSIS*

This dissertation thesis is focused on the role of bacterial tetratricopeptide repeat-like (TPR-like) proteins in the pathogenesis of infection. As a model organism we have chosen highly virulent intracellular bacterium *Francisella tularensis* (*F. tularensis*), whose mechanism of pathogenesis is not completely described.

In the first part of dissertation thesis we took advantage of bioinformatic methods and identified three genes (*FTS\_0201*, *FTS\_1680*, and *FTS\_0778*) with predicted TPR-like domains. Mutants defective in protein expression were prepared by TargeTron insertion mutagenesis. Prepared mutant strains were used for studying the role of selected proteins in pathogenicity and immunogenicity of *F. tularensis* subsp. *holarctica* strain employing *in vivo* and *in vitro* models and further for studying the involvement of these proteins in stress tolerance. Our results showed that the *FTS\_1680* protein is required for intracellular replication and full virulence of bacterium. We also described impaired ability of *inFTS\_1680/200* mutant bacteria to proliferate in *in vivo* system followed by elimination of these bacteria from BALB/c mice organs. Employing proteomic approaches we identified the protein *FTS\_1680* as a membrane associated protein. Moreover ability of mutant bacteria to adapt to stress conditions was tested. We showed that the protein *FTS\_1680* plays an important role in stress tolerance. We also determined the immunoprotective capacity of the vaccination with the *inFTS\_1680/200* mutant against challenge with the virulent *FSC200* strain. The results revealed that the ability of the *inFTS\_1680/200*

mutant to induce an early innate inflammatory response is crucial for its protective potential. Finally, using immunoproteomic approach we defined the profile of *Francisella* membrane proteins recognized by post-vaccination and post-challenge sera and by their comparison we determined novel immunoreactive FSC200 antigens.

The obtained results extend the knowledge of *F. tularensis* virulence factors and thus contributed to possible elucidation of virulence mechanisms. Further, we identified novel immunoreactive antigens useful for a subunit vaccine design.