ABSTRACT

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Title of diploma thesis: Cloning, expression and purification of *Francisella tularensis* disulfide oxidoreductase

Conserved hypothetical lipoprotein called FTT1103 (for *Francisella tularensis* subsp. *tularensis*) is one of the virulence factors of gramnegative intracellular bacteria *Francisella tularensis.* This protein is homologue to protein *E. coli* Dsba. Periplasmatic protein DsbA is member of disulfide oxidoreductase family and it is responsible for disulfide bond formation in newly secreted proteins. The disulfide bonds are essential for right conformation and activity in the proteins. Biotransformatic analysis of protein FTT1103 showed, that this protein contains N-terminal FKBP domain. The domain has probably dimerization activity and it is expected to have chaperone activity too.

The aim of the presented diploma thesis was to verify, if the domain FKBP_N is really responsible for correct function and activity of DsbA protein. Using molecular biology methods we prepared mutant gene with deleted domain FKBP_N (*dsbA*ΔFKBP_N). We cloned this gene into plasmidplazmid *E. coli* pET28b, than we transformed this recombinant plasmidplazmid into *E. coli* cells XL-1. The fused plasmid isolated from grown colonies was transformed into chemocompetetive cells BL-21 using method of heat shock.

The oxidoreductase activity of recombinant protein rDsbAΔFKBP_N was tested by measuring the ability of protein to reduce disulfide bonds in human insulin in the presence of DTT.