

## Abstract

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Title of diploma thesis: The effect of Gp46 on function of HU protein of *Francisella tularensis*

*Francisella tularensis* is a highly virulent bacteria and the DNA binding HU protein is one of its important virulence factors. Recently, it was reported that the Gp46 protein inhibits the function of the *Bacillus subtilis* HU protein (Zhang et al., 2022). The main goal of the presented diploma thesis was to find out if the Gp46 protein of the *B. subtilis* bacteriophage SPO1 also works as an inhibitor of the *F. tularensis* HU protein. Given the high conservation of HU proteins, Gp46 might function as a universal inhibitor of all HU proteins.

At the Department of Molecular Pathology and Biology (Military Faculty of Medicine, University of Defense) neither bacteriophage SPO1 nor its DNA were available, thus, it was initially necessary to acquire the *gp46* gene, which encodes the Gp46 protein, using the overlap PCR method. Subsequently, the Gp46 protein was purified from the *E. coli* expression system using affinity chromatography. In both *in vivo* and *in vitro* conditions, we studied whether and how the Gp46 protein affects the viability and virulence of *F. tularensis*. Using the Electrophoretic Mobility Shift Assay (EMSA), we demonstrated that the DNA binding activity of the *F. tularensis* HU protein is inhibited *in vitro* by the Gp46 protein of the *B. subtilis* bacteriophage SPO1. Next, we showed that the growth of the *F. tularensis* wild-type strain FSC200 expressing Gp46 *in trans* (FSC200/Gp46) matched the growth kinetics of the deletion mutant strain lacking the HU protein (FSC200/ $\Delta$ HU) and differed from the growth of the wild-type strain FSC200. Bacterial proliferation of the FSC200/Gp46 strain within bone-marrow derived macrophages was comparable to that of the deletion mutant strain FSC200/ $\Delta$ HU, with the replication efficiency of both strains being significantly reduced compared to the replication efficiency of the wild-type strain FSC200. These results, combined with findings from a

recently published study on the Gp46 protein, suggest that the Gp46 protein could be a universal inhibitor of HU proteins across bacterial species.

**Keywords:**

*Francisella tularensis*, tularemia, virulence, HU protein, Gp46