Spr0334, new protein of cell division in *Streptococcus pneumoniae*

Streptococcus pneumoniae is an important human pathogen. The geonome of this bacteria encodes a single gene for eukaryotic-like serine / threonine protein kinase called StkP. StkP regulates many physiological processes such as pathogenesis, competence for genetic transformation, resistance to various stresses and resistance to antibiotics. It also affects the transcription of many genes involved in cell wall biosynthesis, pyrimidine metabolism, DNA repair and iron uptake. Recent studies have shown that StkP is located in the cell division septum and significantly regulates cell division and morphology. Its substrates include, among others, cell division protein DivIVA, FtsZ and FtsA.

Analysis of phosphoproteome maps of wild type and Δ*stkP* mutant strain of *S. pneumoniae* showed that *in vivo* StkP phosphorylates several putative substrates including the protein Spr0334. Mass spectrometry analysis identified phosphorylation sites of the protein Spr0334: threonine 67 and threonine 78. Furthermore, it was found that the protein Spr0334 is located in the cell division septum, which led to the hypothesis that it could be newly identified cell division protein in *S. pneumoniae*.

The main aim of this thesis was to describe the function of the hypothetical protein Spr0334 in *S. pneumoniae* and determine the effect of phosphorylation on its activity. By deletion of the gene *spr0334*, we created a mutant strain of *S. pneumoniae*, which we further characterised. We found out that deletion of the gene for protein Spr0334 results in production of small round cells with frequent presence of minicells that often do not contain any DNA.

Furthermore, we prepared constructs for complementation of *spr0334* deletion in *S. pneumoniae*. We created complementation strains of *S. pneumoniae* carrying wild type or mutant allele of the gene *spr0334* under inducible zinc-promoter in dispensable *bgaA* locus. We performed amino acid exchange of T67 and T78 for neutral alanine, thus creating phosphoablative form of Spr0334, which can not be phosphorylated. We studied the expression and phosphorylation of both, native and phosphoablative, forms of Spr0334 in *S. pneumoniae* and we confirmed that the amino acids T67 and T78 are phosphorylation sites of protein Spr0334 *in vivo*. Phenotypic studies of these strains showed that the phosphoablative protein Spr0334 is active and can compensate for *spr0334* deletion. Thus, phosporylation is not essential for Spr0334 activity.

Finally, we created strains of *S. pneumoniae* carrying fusion gene *gfp-spr0334* under inducible promoter in dispensable *bgaA* locus. Using fluorescence microscopy, we observed localization of this protein in the wild type background, in strain Δ*spr0334* and in strain Δ*stkP*, in which substrates of StkP are not phosphorylated. We found that protein GFP-Spr0334 is localized predominantly in the cell division septa, and this localization is not significantly altered depending on its phosphorylation state.

In conslusion, our findings indicate that protein Spr0334 is a cell division protein that affects cell morphology and participates in selection of cell division site and/or chromosome segregation in *S. pneumoniae* by yet unknown mechanism.