Protein phosphorylation by protein kinases is a key mechanizm that enables both eukaryotic and prokaryotic organizm sense and read environmental signals and convert these signals into changes in gene expression and thus proper biological response. One of the main phosphorylation systems in bacteria consists of eukaryotic-like Ser/ Thr protein kinases.

The genome of human pathogen *Streptococcus pneumoniae* contains single Ser/ Thr protein kinase StkP. StkP regulates virulence, competence, stress resistance, gene expression and plays an important role in the regulation of cell division cycle. Analysis of phosphoproteome maps of both wild type and Δ*stkP* mutant strain of *S. pneumoniae* showed that *in vivo* StkP phosphorylates several putative substrates including the cell division protein DivIVA (NOVÁKOVÁ *et al*., 2010). DivIVA in *S. pneumoniae* is localized at midcell and at the cell poles. It was proposed to be primarily involved in the formation and maturation of the cell poles (FADDA *et al*., 2007).

The aim of this thesis was to investigate phosphorylation of the cell division protein DivIVA in *S. pneumoniae*. Gene *divIVA* was cloned, expressed in *E. coli* and protein was purified via affinity chromatography. Phosphorylation of DivIVA by StkP was examined in a kinase assay. We confirmed that DivIVA is a direct substrate of StkP *in vitro*. Further we prepared a set of mutants in *divIVA* gene. We replaced two presumable phosphoaminoacids Thr15 and Thr201, identified by mass-spectrometry with inert aminoacid Ala, thus producing phosphoablative mutants. Phosphoablative proteins contain no phospho-accepting amino acid residues and cannot be phosphorylated. Using these mutants we confirmed that DivIVA is phosphorylated by protein kinase StkP at Thr201 and with lower efficiency at Thr15.

We prepared a construct for complementation of *divIVA* deletion in *S. pneumoniae*. We constructed strains of *S. pneumoniae* expressing phosphoablative and phosphomimetic mutants of *divIVA* under inducible zinc-promoter. Phosphomimetic DivIVA´s carry mutation Thr15Glu and/or Thr201Glu. Presence of negatively charged Glu mimics phosphorylated state of protein. We studied expression and phosphorylation of DivIVA mutated proteins in *S. pneumoniae* and we confirmed that Thr15 and Thr201 are phsophorylated *in vivo*. Our phenotypic study showed that phosphorylation of DivIVA is not essencial for its function. On the other hand our data suggest that hyperphosphorylation inhibits DivIVA activity.