

Phosphoglucosamine mutase (GlmM), an enzyme taking part in biosynthesis of cell wall, has been recently proven to be essential for *Streptococcus pneumoniae*.

The main goal of this thesis was to prove *in vivo* that GlmM serine residues S99 and S101 phosphorylation is essential while the necessity of it was already proven indirectly based on transformation efficiency. For this purpose we have prepared a strain with two copies of the *glmM* gene – the first one with amino acid changes on monitored serine residues located at native locus; and the second ectopic copy of the wild allele of *glmM* gene under control of inducible zinc promoter. We have observed morphology, growth, and GlmM expression with and without the presence of an inductor. All the observed parameters show that the cells are not viable without ectopic *glmM* expression, thus the essential protein GlmM is functional only when phosphorylated on S99 and S101 residues. Further, we have attempted to localize the enzyme in the *S. pneumoniae* cell. We have fused GlmM with fluorescent marker GFP and by using the fluorescent microscopy we have proved that GlmM is cytoplasmic protein.

Another goal of this thesis was to find an unknown third phosphorylation site of the GlmM protein which is dependent on the protein kinase StkP. From *in vitro* kinase assay and subsequent MS analysis, it was evident, that apart from residues S99 and S101, GlmM protein is phosphorylated at positions T304, S414, T416, and T438 by protein kinase StkP. Moreover, GlmM protein is capable of an autophosphorylation on serine residues S99 and S101. These newly described phosphorylation sites will be the topic of the further research.