Summary

Bacillus subtilis is considered a model organism for research on bacterial cell development during its cell cycle. Under conditions unfavourable for vegetative growth, this Gram-positive bacterium starts an alternative developmental pathway. In sporulation process *B. subtilis* forms an endospore which, upon the release to the environment, guarantees the survival of bacterial genetic material. Thanks to its complex structure, endospores are extremely resistant to harsh environmental conditions. As dormant entities spores are able to survive for years. Seemingly inactive, endospores constantly monitor the environment, expecting improvement in conditions. During the process of germination dormant spores transform into a vegetative form, which will continue the active growth. The best known pathway of germination initiation so far is the nutrient-dependent initiation by germination receptors, with GerA considered an independently acting receptor. GerA reacts to the presence of amino acids L-alanine or L-valine in the environment. Based on the protein encoding sequences it is known that the receptor consists of 3 proteins: A, B and C. Due to their complex structure and transmembrane features, structural and functional data about the germination receptors are still very limited.

Objective for this dissertation was to investigate given aspects of GerA receptor functions and to propose a possible mechanism of action for the entire protein complex in germination initiation.

First, there was made an attempt to construct a strain of *B. subtilis*, in which all of the germination receptor's proteins could be detected and visualized. Despite constructing the strain with given antigen epitopes cloned to each gene from the *gerA* operon, endospores obtained from such a strain were unable to germinate. Next, the research focused on conserved motif VPFP analysis in protein A. In order to do that substitution of proline in position 324 (P324S, P324G, P324A, P324F) was performed. Obtained strains were analysed for the properties of their spores during germination process, in the presence of both activators (L-alanine, L-valine, AGFK, dodecylamine) and an inhibitor – D-alanine. Microscopic and gemination analyses showed that substitution in position 324 of protein A created a heterologous population of endospores with changed properties, in comparison to endospores from wild type strain. First of all, it was observed as spores in phase dark or grey before even the germination was initiated, which suggests a hydration of their core compartment during the formation process. It was also connected with a reduced sporulation efficiency of strains with mutations. Moreover, endospores with mutations P324S and P324G were also able to germinate in the exclusive presence of inhibitor. Because the endospore germination efficiency of analysed strains varied, in the end operon *gerA* genes expression analysis was performed. The results showed that the differences observed on the level of germination efficiency could be caused by the uneven level of operon genes expression, especially when the original genes arrangement was disturbed.

The results obtained from the research presented here suggest that the initial genes arrangement in *gerA* operon might be crucial for the efficient protein synthesis to functional GerA protein complex. It could also explain why endospores from strain with antigen epitopes in *gerA* operon have not germinated. What is more, results from mutagenesis of protein A's position 324 indicate that VPFP motif plays essential role in the function of the entire GerA complex, probably as a modulator for protein B, where a putative ligand binding pocket is located. In the light of recently published data on GerA receptor proteins, results from position 324 mutagenesis could be an important argument in determining the function for protein A in the entire receptor activation.