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GerAA protein phosphorylation as a possible mechanism regulating the activity of GerA germination receptor of *Bacillus subtilis* endospores

Bacillus subtilis is a Gram-positive bacterium commonly found in soil. In the unfavorable environmental conditions it can form **metabolically dormant endospores** which are resistant to many harsh conditions such as high temperature, desiccation and UV radiation. In this dormant state spores are able to survive for many years. After being exposed to the appropriate stimuli, called germinants, they start to **germinate** and return to their vegetative state. The exact molecular mechanism of this complex process is not established yet. However, it is well known that initiation of *B. subtilis* endospore germination can be induced by specific nutrients. Thanks to the presence of a specific inner membrane protein, called **GerA receptor**, spores can germinate in response to L-alanine or L-valine.

GerA receptor is composed of three subunits – **GerAA**, GerAB and GerAC proteins (encoded by one polycistronic operon). Comparative genetic analysis of different reference laboratory *B. subtilis* strains conducted in our laboratory revealed that there are **three variants of GerAA protein varying in amino acid residues at positions 299 and 302** [unpublished data]. One of these variants contains threonine (Thr) at position 299 and serine (Ser) at position 302 and is synthesized in *B. subtilis* 168 strain. These are the **amino acids being potentially phosphorylated in bacterial cells**. In the same laboratory strain (*B. subtilis* 168) used for research in French laboratories (in this case nucleotide sequence of *gerAA* gene comes from the French internet database of Pasteur Institute – http://genolist.pasteur.fr/SubtiList/index.html) two residues on which phosphorylation doesn't occur – alanine (Ala) and proline (Pro) at 299 and 302 positions, respectively – are present. **Kinetics of L-alanine-dependent germination** of the spores with 299Ala/302Pro GerAA is considerably altered in comparison to endospores with 299Thr/302Ser GerAA [unpublished data]. The third GerAA protein variant (299Ala/302Ser), present in *B. subtilis* PY79 laboratory strain and *B. subtilis* 3610 environmental strain, does not influence the rate of spore germination in comparison to endospores with 299Thr/302Ser GerAA. Our results show that integration of *gerAA* gene encoding 299Thr/302Ser or 299Ala/302Ser GerAA into *amyE* locus of *B. subtilis* 168 strain (with GerAA 299Ala/302Pro) restores the spores' ability to germinate in response to L-alanine [unpublished data].

Taking into account that the state of the protein's phosphorylation is one of the possible factors that can modulate proteins' biological activity and consequently change the phenotype of the cells in which these proteins are synthesized, **we hypothesize that phosphorylation of GerAA protein (a subunit of GerA germination receptor of *B. subtilis* spores) has an influence on the receptor's activity**. Therefore the main goal of my future work is the analysis of phosphorylation of GerAA protein *in vivo*. In addition, the influence of different substitutions at 302 position of GerAA protein on spore germination kinetics will be assessed.