Variants of GerA receptor in endospores of different *Bacillus subtilis* laboratory strains.

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Bacillus subtilis is a Gram-positive aerobic bacterium able to form metabolically dormant endospores in disadvantageous environmental conditions. For years laboratory strains of this species have been used as model organisms in basic research focusing on, e.g. sporulation and bacterial spore germination.

B. subtilis endospores are resistant to many unfavourable conditions, thanks to which they can sustain for years in the environment without loosing the ability to germinate. Spore germination is initiated by the appearance of specific inductors in the environment, so called germinants. Among different ways of induction, germinants trigger germination through germinant receptors (GerA, GerB and GerK). While the presence of L-alanine or L-valine initiates spore germination through GerA germination receptor, both GerB and GerK are necessary for germination triggered by AGFK mixture.

The following work has been focused on the analysis of *B. subtilis* GerA germination receptor functioning. Function of GerA receptor is strongly conserved among different laboratory and environmental strains of this species. However, two *B. subtilis* 168 variants which endospores respond differently to L-alanine in terms of germination were found in the strain collection of Laboratory of Molecular Bacteriology of Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk. While 168G spores are able to germinate in the presence of this germinant, GerA receptor from 168F spores, in the same conditions, is not functional.

Comparative genome analysis of selected *B. subtilis* laboratory strains (168G, 168F, PY79 and PS832) pointed the presence of two single nucleotide polymorphisms (SNPs) in *gerAA* gene encoding the A subunit of GerA germination receptor. These SNPs are responsible for existence of the following GerAA protein variants, which differ in positions 299 and 302 of their amino acid chains: 299Thr/302Ser (168G), 299Ala/302Pro (168F) and 299Ala/302Ser GerAA (PY79, PS832).

Spore germination analysis of different *B. subtilis* strains showed that different GerAA protein variants are responsible for the differences between 168G and 168F spore germination phenotypes in the presence of L-alanine. Change of the residue at 302 from Ser to Pro is responsible for the mentioned discrepancies. On the other hand, different residues at position 299 of GerAA influence the rate of spore germination and to some extent – the efficiency of this process.

The main goal of the following work was to find the reason for different functionality of individual GerAA variants. Spore germination analysis of newly constructed strains, together with *in silico* analysis of GerAA protein structure suggest that: i) Ser302Pro change may influence the secondary structure of GerAA protein; ii) function of GerA germination receptor is not regulated by phosphorylation of GerAA's 302 residue; iii) different GerAA variants have different affinity to other subunits of GerA germination receptor.

In silico analysis of GerAA protein showed that 347Arg is a potential candidate for germinant (L-alanine) binding at the beginning of spore germination. Due to the strong alkaline character of the residue in the depicted position, spore germination analysis of the strains carrying different GerAA variants with gradually decreasing 347 residue alkalinity was performed. Results obtained during analysis showed that the alkaline character of 347 residue plays an important role in GerA receptor functioning.