

Review of: "Visualization of SpoVAEa protein dynamics in dormant spores of *Bacillus cereus* and dynamic changes in their germinosomes and SpoVAEa during germination"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

The manuscript, untitled "Visualization of SpoVAEa protein dynamics in dormant spores of *Bacillus cereus* and dynamic changes in their germinosomes and SpoVAEa during germination" aim to describe the mechanisms of *B. cereus* spore germination by the observation of the movement of specific proteins, involved in germination process, and their interactions.

The present work is of high quality and of great interest to the field. The authors remain cautious, to their credit, with their results because they are based on the observation of plasmid-modified strains. Nevertheless, the results obtained in this study allow a better understanding of germination mechanisms and this type of results can provide elements to have a better control on the development of the spore flora.

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My first main comment concerns the statistic tests. I think the tests that are used in the different section of this work must be clarified. For example, l.160-162, the statistical test is not provided and the "significant" difference, referring to the graph in Figure 3, does not appear clearly to me (I would say that, just given the figure, the difference is not significant). An effort should be done on this aspect.

A second main comment concerns a matter of vocabulary. I am not sure to fully understand the expression "germination efficiency". Is it a matter of time of germination, or a matter of rate of germination? Or both? "Germination efficiency" appears several time but the authors seem to give a particular attention to germination duration. For example, l.335-336 "germinated more slowly". Does it refer to a "less efficient" germination? Maybe the authors can clarify "germination efficiency".

Finally, I did not see the number of analyzed spores for some experiments. As the results are provided as mean \pm SD, it is of interest to know the number of analyzed spores.

Minor comments or suggestions

l.15 (introduction) maybe change "to control *B. cereus* spore development" or so.

l.91 add "red fluorescent" before "mScarlet-l" (to be consistent with "green fluorescent protein (SGFP2)")

l.239-245 It is not clear to me as the authors firstly say that "intensity of germinosome FRET foci fell significantly compared to the value at time 0 min" (l.240) but secondly that "NFRET values at 10, 20, 30, 40, 50 and 60 min are not significantly different from value at 0 min", both for strain F06. Please clarify.

l.246 "population averages" how many spores the population contained?

Figure 8. Does "germ cell wall" refers to coats+cortex?

l.373 (the caption of Fig. 8 in general) why do the authors refer to GFP and RFP here? Does this refer to the fluorescence channel used? It is the only mention of GFP and RFP in the manuscript.

Material and Methods. Do the authors can provide the bandpass characteristics of the emission filters?

l.452 Can the author clarify what is the aim of the heat treatment (15 min at 70°C)?

l.455 Please clarify or explain "OD₆₀₀ of 15", to me this value has no sense (maybe 1.5?)