

Interactive comment on “Freezing from the inside. Ice nucleation in *Escherichia coli* and *Escherichia coli* ghosts by inner membrane bound ice nucleation protein InaZ” by Johannes Kassmannhuber et al.

Anonymous Referee #2

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The study of Kassmannhuber et al. (2019) is essentially about the ice nucleation activity of manipulated *E. coli* and its bacterial ghosts with the ice nucleation active protein InaZ (from *P. syringae*) embedded into the inner bacterial membrane. The concept of bacterial ghosts is very interesting. The study comprise a plausible procedure and a comprehensive literature research. However, I suggest to reject the paper in its current form for two main reasons:

(a) The study does not include any statements about its importance or its implication for the atmosphere. The main objective of Atmospheric Chemistry and Physics is to inves-

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tigate the Earth's atmosphere including all relevant chemical and physical processes. As the link to the atmosphere is not given at all in Kassmannhuber et al. (2019), the study does not fit to the objectives of ACP.

(b) The study itself is not clearly motivated. What is the reason for the artificial generation of bacterial ghosts with embedded ice nucleation active proteins in the INNER membrane? I do not think that it is sufficient to justify the study by the fact that no one has done it before (page 3 line 41-42). An elaboration of the advantages of the new approach are essentially necessary in particular in comparison to a previous study in which the ice nucleation active protein was embedded in the outer bacterial membrane (Kassmannhuber et al. 2017).

The results of the current study are worth for publishing when the above listed points will be addressed satisfactorily. If it is not possible to relate the results from this study to atmospheric processes, I suggest to publish the work in a more microbiology related journal.

Specific comments:

Although there is no real general rule (an attempt was done in Vali et al. 2015) and things can be defined differently, the applied terminology of ice nucleation is very untypical, which makes the reading of the paper hard for experts. In the following, I list some examples:

- INP is used for ice nucleation protein instead of ice nucleating particle
- IN for ice nucleation instead of the already old term ice nuclei
- Freezing or ice nucleus spectra is usually a derived quantity (Vali 1971), it is not clear what fice (Fig. 3A) means and it is defined

For good scientific practice, it is necessary to explain the uncertainties of experiments (page 8 line 10: “Error bars represent the standard errors.”).

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As the fraction frozen (explained in Vali et al. 2015, probably fice in the current study) and hence also T50 is a function of number of ice nucleating particles (Augustin et al. 2013), it is not appropriate to compare T50 values of different samples having different number of ice nucleating particles. It is better to use conservative quantities such as the ice nucleus spectra which is normalized to mass, number etc.

Technical corrections:

The paper is written using a very untypical structure. Please revise the paper according to instructions given at https://publications.copernicus.org/for_authors/manuscript_preparation.html . The citation style is incorrect. Usually the citation brackets have to prior to the punctuation mark. I am not sure if it is a general law, but to appreciate older studies the older paper are given first, i.e. the order is from old to new.

References:

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