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Biogeosciences Discussions

Interactive comment on "Evaluating the potential for Archaea to serve as ice nucleating particles" *by* Jessie M. Creamean et al.

Anonymous Referee #3

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Ice nucleation activity of biotic material is an interesting topic and investigation ice nucleating properties pf Archaea is an interesting approach. However, the problem with this study is that I do not feel that there was well thought out experimental design. 1. The authors did not treat all four organisms the same, comparing different dilutions, intact cells from one species vs lysed cells from another. 2. The authors argue correctly that, once airborne, halophiles would be exposed to a dilute environment. However, the salinity of the selected dilution is not representative of cloud droplets. Moreover, why intact cells that did show ice nucleation activity where not further diluted to cause cell lysis, and conversely, cells that were lysed were not tested at lower dilutions to keep cells intact is unclear. 3. The authors reported that diluting the media reduced survivability of halophiles and, in order to account for that, they determined the number

of intact cells by microscopy. Yet, cell numbers reported in Table 1 are simply derived by multiplying cell numbers by the dilution factor (Table 2), and thus, the study does not account for any losses caused by the dilutions. Undiluted cell suspensions should have been fixed to avoid cell lysis and counted. How can the authors account for a combined effect of intact cells and lysed cell material in these assays? 4. The purpose of 30% H2O2 treatment is to determine the contribution of abiotic factors. However, the authors question the efficiency of the digestion protocol. If these 'digests' are a mixture of biotic and abiotic compounds, then there is no need for including the data. 5. There is no statistical evidence presented that any of the organisms/treatments/controls were different albeit 24 replicates.

As intact cells for H. walsbyi and N. pharaonic were not investigated and lysed cells did not show ice nucleation activity, they do not contribute to the study and should be removed.

Methodology: L116: It is confusing and unclear why it is relevant the cells were first grown at the College of Charleston and then shipped to Colorado State University. L116: Unclear why cells were first grown to mid-log phase but subsequently to somewhere during log-phase. L117: Please provide more detail no monitoring cell density. What microscope, cell counts, add reference on microscopy-cell abundance procedure. Also, the objective was most likely a 100 x with an extra 10x magnification within the eyepiece or camera. Unclear why cell density would be of relevance prior to shipping? L117: reference to Table 1: cells were shipped to a different university and additional experiment where performed at this university I assume. Cell numbers always remained constant during shipping, storage, and time passed until experimental setup? Table 1: Is salinity presented as gram NaCl? I am asking because e.g. DSMZ media 97 contains 250 g NaCl. Should this be 250 ppt and 25%. L127: If the current study truly determined the lysis thresholds, why did the authors not in include dilutions for H. walsbyi and N. pharaonic. Cells were diluted 1:15 one time not serially. Dilution resulted in the lysis of two out of four selected organisms. Reason why not includ-

ing lower dilutions that left cells intact as well as including dilutions for the other two organisms that would have resulted in cell lysis is unclear; particularly since the authors determined lysis thresholds. L128: Why were cells grown again if they already reached the desired cell density prior to shipping (L117)? Ll29: What was the reason for selecting two more dilutions for H. morrhuae but not the other three organisms? It is well known that cell density has an effect on ice nucleation. L130: add detail on microscopy. L138: what was the coolant? L142-148: (i) Heat treatment and 30% H2O2 amendments were intended to determine the effect lysed cell material and inorganic molecules on ice nucleation. If the authors think that the digestion was ineffective, they should have altered the protocol rather than hinting at the need for it. (ii) a general problem throughout the experimental design is the comparison of different cell 'material' i.e., intact, lysed, intact/digested, lysed/digested. It appears that the initial intact vs. lysed was an artifact and the authors went with it, but essentially, the study compares apples and oranges.

Results and discussion As the results and discussion section will largely change after removing a large portion of the dataset here are some general comments. When did controls freeze? H. morrhuae is atypical compared to all Archaea or the ones investigated? L167-172: Irrelevant as lyses cells are compared to intact cells. This is a study on INPs. No sure why it is 'interesting or relevant' to discuss cell lyses. As mentioned before low dilutions should have been used to not lyse cells. L176-175: H2O2 treatment should provide information in the abiotic fraction not organic. L174-: Are any of these reported changes in freezing temperatures statistically different from intact cells? L183-186: Discussion on ineffective H2O2 treatment. Effectively separating or removing specific fractions when investigating ice nucleation properties is essential. This section does not strengthen the manuscript. L192-193: Why is N. pharaonis now only partially lysed contradicting previous statements?

Conclusion: L209-213: seems more suited for an introduction.

Figure 1: Please remove the shading. The reader gets the impression that the area

under the curve is of importance. Why on a log scale?

Figure 2: Why are there no controls for the dilutions 1:6 and 1:30? As shown in Table 2 the salinity ranges from 2.7%-0.5% in these samples.

Other edits: Check for missing punctuations L10: delete 'from microorganisms' L15-16: change 'of a subset of archaeal cells from Haloarchaea' to 'selected genera of the class Haloarchaea' L16-18. Reason for comparing intact cells two lysed cells from different genera is unclear L17: without comparing to the freezing temperature of an abiotic control, I would not consider -18C warm. Please rephrase. L18: What are warm temperature INPs? Please rephrase. L23: 'necessary to improve'. These are extremely strong words. How about 'intriguing' L40: delete ' approximately' L54: replace 'however' with 'further' L57-61: please split this running sentence. L64: Do all minerals except for feldspars function as INPs? I suggest deleting 'aside some feldspars' L71: delete 'relatively' L82-83: Are there some bacterial cell that produce no peptidoglycan as the authors say 'nearly ubiquitous'? L93: 'possess' L96: replace 'it is' with 'its' L97: For the other three genera cell characteristics were briefly described as a justification to include them in this study. Why not for Natronomonas? L100: change to 'they are relatively easy to culture compared to other archaeal lineages' L105: What do the authors mean by 'halophiles and hypersaline' L106-109: I recommend removing this paragraph. This paragraph is trying to oversell the importance of this research. L116: delete '(i.e., midway through the period of exponential cell growth)' L118: delete '(i.e., the period characterized by cell doubling)' L141: delete 'for all species during each experiment.'

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