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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 #2

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There have been no data available on the ice nucleating activities of archaea, and this study examined the capacity of four haloarchaeal species with different cell wall types to serve as INPs. In general, the cells that remained intact after dilution in distilled water incited freezing at the warmest subzero temperatures observed, and additional experiments provided evidence that the activity is mediated by a proteinaceous or organic compound associated with the cells. I suggest a minor revision for the title by replacing "haloarchaea" for archaea since that was the only type of archaeal species they tested and more accurately describes the study. Below are more detailed and specific comments to consider when revising this manuscript.

Abstract, Lines 19-22: As written, this sentence implies that thermophiles are prevalent "in other cold niches", which is not where one might expect thermophiles to be

prevalent.

Abstract, Lines 22-24: Since the ability of archaea to "become airborne" or "impact cloud formation" was not examined in this study, please consider revising this closing statement in the abstract to more directly reflect the results obtained and their implications.

Line 74: Suggest revising this statement to "up to 40% of the microbial taxa in an ecosystem".

Lines 118-119; 132-133: That fresh cultures were sent overnight is described, but please indicate how much time passed between receiving the cultures at CSU and performing the ice nucleation assays. This is very important information needed to evaluate the results because it is well established that the phase of growth and culture age affect ice nucleation activity in bacteria (e.g., Nemecek-Marshall et al. 1993, J Bacteriol 175:4062–4070; Fall and Fall 1998, Curr Microbiol 36:370–376; Yankofsky et al. 1983, Current Microbiology 9:263–267).

Lines 122-123: This sentence describes a result and is out of place in the methods.

Lines 124-126; 147-148: These sentences in the methods would be more appropriate for the discussion section.

Line 128: Please clarify what is meant by "active". Do you mean ice nucleation active? Metabolically active?

Lines 145-147: Please provide more detail on how the UV exposure was done to irradiate the liquid samples. If this was done by exposing a sample held within a test tube and since UV is opaque to most plastics as well as being attenuated by water and particulates (cells), it is important to explain the composition of materials involved and procedure in more detail (e.g., dose rate of UV source, distance of samples from the source, and if the dense suspension was mixed during exposure). Please also indicate the final concentration of peroxide used in the experiments in v/v. For example, if 0.75

mL of 30% H2O2 + 1.5 mL aliquot of suspension = 2.25 mL, so was it 7.5% H2O2 v/v? Finally, are all the haloarchaea used in this study catalase negative?

Line 163: Please clarify what is meant by “markers” and note that polysaccharides are ubiquitous components of archaeal and bacterial cell envelopes.

Lines 168-169; 171-172: Please consider revising line 168 to state that “lysed cells” of these species do not have ice nucleation activity. The authors should also consider mentioning that it is well known that lysing of ice nucleation active bacterial cells decreases the efficiency at which they are INPs (e.g., Lindow et al. 1989, Mol. Plant-Microbe Interact. 2, 262). Are there any data available from experiments with lysed cells of Halococcus morrhuae and Haloferax sulfurifontis? It would not be surprising if the lysed cells

Lines 176-179: Figure 2 indicates that INPs active at the warmest temperatures were heat labile, so I’m confused by what is meant by a "more substantial amount" of something else. Since the fraction of samples that froze at each temperature is known, this can be used to calculate the number of INPs at each temperature according to the method of Vali (1971, J Atmos Sci 28:402–409). These data provide context for inferring the fraction of the cell populations that were ice nucleation active at a given temperature/experimental condition.

Lines 182-184: When catalase was added to samples of the less dilute cell suspensions, were oxygen bubbles observed/produced? I follow this argument, but it has me wondering about the “residual organic material” statement. Are the authors suggesting that treatment of the cell suspensions with peroxide oxidizes all macromolecules and organic constituents of the cells completely to CO2?

Lines 187-188: I think this section is talking about Figure 3, but on closer inspection, I don’t see Figure 3 referred to in the main text.

Lines 188-190: Please explain how these different behaviors should be interpreted with

respect to the properties that can be inferred from the archaeal INPs.

Lines 198-199: I would not describe the salt concentrations used in these experiments as “low”, at least not in comparison to rain, snow, or freshwaters. The average concentration of salt used in the assays was $\sim$1% and is roughly a quarter seawater. Please can the authors describe conditions that would allow cloud droplets to achieve such high ionic strength.

Lines 205-206: Please expand on this point as I am not aware of any work that has shown similarities in motifs between gammproteobacterial IN proteins and S-layer proteins.

Figure 3: not mentioned in main text.

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