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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Effects of atmospheric conditions on ice nucleation activity of *Pseudomonas*" *by* E. Attard et al.

Anonymous Referee #2

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This article provides much needed measurements of three atmospheric strains of bacteria as IN. Advantages of this work are that the bacteria are isolated directly from cloud and glacier water, and thus accurately represent those found in nature. Also, changes in IN activity due to exposure to various modifications which may occur in atmospheric aerosols and may cause the death of the bacteria are studied. The topics studies are ambitious. In some instances, additional measures in the form of control experiments, need to be taken to ensure correct interpretation of the results. These are discussed below:

Pg 9496, it is stated that bacteria suspensions were prepared in sterile water to obtain 5 x 10ˆ8 to 5 x 10ˆ9 cells per ml. How was this concentration determined? Also this is an entire order of magnitude in concentration. Differences within this concentration

range could seriously impact the results and conclusions. Please explain if and how the impact of concentration was addressed?

Pg. 9497. Of the numerous compositions of IN in the atmosphere, the authors choose to compare biological IN activity to that of a single mineral compound, montmorilonite. This is not an obvious or necessarily common mineral IN. This choice of comparison should be justified.

Bacteria were placed in distilled water which is likely to split apart the cells and may release proteins and other materials from within the cells. Were any control experiments in other solvents or the original glacier melt water or cloud water conducted? Do the authors have any way to access bacteria breakup and counting of fragments? This issue should be addressed through additional control experiments.

Testing the effects of pH is an interesting is a worthy experiment. However, orchestrating an appropriate experiment is a challenge. The authors choose to add sodium acetate and acetic acid to the samples to create the buffers. What is the solubility of acetic acid at temperatures below freezing? The solubility of some organic acids is greatly reduced at colder temperatures. If this is the case with acetic acid, then the presence of acid itself may facilitate freezing. Control experiments should be conducted in buffer solution only, without any bacteria. The authors say that they have corrected for colligative effects following Koop and Zobrist , 2009, but I do not think that that method is directly applicable to the solutes used in this study, and regardless, is an estimation, whereas running control freezing experiments would be direct evidence of the effect of the buffer chemicals.

The data is figures 1-3 is only displayed down to -10 C. Pure water should freeze well below that temperature ($\sim$-38 according to the authors), but no results proving that water behaves in this manner is included. In drop freezing experiments such as these, it is necessary to confirm that the substrate is not interfering with the experimental freezing temperatures. Do pure water droplets freeze at -10 on this substrate? If so,

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the bacteria are having no effect whatsoever. I don't think this is likely, but it must be confirmed through control experiments.

For ease of reading, the caption for figure 3 should include definitions of ns, a, and b. Definitions should also be included in figure 4 figure 5, including ones for *, a* and b*.

Interactive comment on Atmos. Chem. Phys. Discuss., 12, 9491, 2012.