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Interactive comment on “Immersion freezing of ice nucleating active protein complexes” by S. Hartmann et al.

Anonymous Referee #1

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Hartmann et al. use the now well known and highly capable LACIS flow tube to study the ice nucleation efficiency of SNOWMAX, a commercial ice nucleating bacteria used in e.g. artificial snow production. The effect of bio-aerosol on ice nucleation has been the focus of many recent laboratory studies and SNOWMAX has now been well characterized by a number of groups; previous work is well referenced here. The novelty of this manuscript is therefore not in the freezing behavior of SNOWMAX - which would not, by itself, warrant another publication in a journal such as ACP since it has been done frequently before - but instead is to place the results within a framework that tries to understand freezing based on the number of (ice nucleation active) “complexes” present within droplets. Unfortunately the physical nature of what a complex is isn't fully developed in this manuscript, nor is if complexes are units of bacteria inherent to a commercial product (which SNOWMAX is), a by-product of laboratory generation, or

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– as the authors appear to assume – a parameter relevant to the atmosphere. This, more than anything, must be corrected before possible publication.

That being said, this paper may be publishable in ACP after major revisions. As stated, the definition of “complex” remains undefined and certainly is not a value that has ever been measured in the atmosphere. My first suggestion is to provide an exact meaning of what a “complex” is : in the manuscript I find assertions that it is bacterial fragments over 100 nm in size, it is a portion of a cell wall that promotes ice formation, and it is confused with the onset temperature of ice nucleation of different proteins (so-called types A, B, C of Turner et al. 1990). The reader is currently unable to interpret if a “complex” is related to the production method of SNOWMAX (i.e., is it a bacterial fragment of a certain size related to the production method – grinding - to maximize ice nucleation?) or is it a result of the preparation of the bacteria in the lab (a by-product of the droplet production method)? How is the abundance of complexes actually relates to atmospheric biological material? This will need to be discussed at length in revision. I further note that there are several statements made about differences between this and the work of Moehler and agreement with Wood. I wonder – and would like the authors to comment – if this might be due to different samples of SNOWMAX with different properties. For example, what if SNOWMAX is sometimes ground to one size distribution and sometimes another? Can this be precluded? My suggestion here is for some off-line size distribution work to be done along with some contact with the company to provide data.

Also, the authors try to extrapolate their freezing model “CHESS” to atmospheric cloud modeling (statements are made in the abstract and summary). Many critical steps are missing between the lab model and cloud, however, and these are never actually discussed. As it stands the statements seem to be used to increase the importance of this model by suggesting – but never supporting – this assertion. The “missing steps” include the abundance of bacteria in the atmosphere, the fraction that are ice active, and how the “complexes” discussed in the paper related to e.g. bacterial fragments

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in the free atmosphere. If the authors want to maintain this possible link from CHESSESS to cloud parameterizations these intermediate steps must be addressed in the revised manuscript; it is not sufficient to simply suggest there may be a possible use here.

Otherwise, I find this a well written paper with reasonable length and high quality figures. Since much of the data is already found in the literature the aforementioned points absolutely must be addressed if this is to be ultimately published in ACP otherwise this paper is a repeat of previous measurements, albeit with a new chamber.

Some specifics on major point 1: e.g. Abstract (Line 10): “The experiments performed in the lower temperature range, where all droplets freeze which contain at least one INA protein COMPLEX, are used to determine the average number of INA protein complexes present, assuming that the INA protein complexes are Poisson distributed over the droplet ensemble.” (complex is my highlight) Surprisingly, the term “single complexes” is never fully defined nor is how they relate the atmosphere? It seems to me that these “complexes” are actually the smallest unit of bacteria IN THESE STUDIES which may not have a bearing on the atmosphere (that is to say the authors haven’t addressed what a complex is, how it is produced, what its physical properties are). Relation to other studies likely means they are related SNOMAX properties, or perhaps lab conditions, not atmospheric relevance. To further this point the figures and text appears to indicate a complex is ~ 100 nm in size (i.e., particles smaller than this are devoid of ice nucleation). This is certainly not the size of a protein nor is it the size of a bacterium. It might be the size of some features on cells which promote ice nucleation but this is never defined. What is it then, a fragment of a bacterium? What causes such fragments in SNOWMAX? Is it industrial preparation to maximize ice nucleation efficiency? Is this related to actual bacterial fragmentation in the atmosphere or a byproduct of industry? Are such fragments always 100 nm in size or sometimes different depending on preparation? If the later then this is NOT how one would want to parameterize atmospheric ice nucleating bacteria. Central point: why should we consider “complexes” representative of the atmosphere and not simply this experiment?

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More on major point 2: e.g. Abstract (20): “The results obtained in this study allow a new perspective on the interpretation of immersion freezing experiments considering INA protein complexes and the derived simple parameterization of the heterogeneous ice nucleation rate can be used in cloud resolving models for studying the effect of bacteria induced ice nucleation.” (this is repeated in summary). This is a huge leap from lab to atmosphere. Indeed, the authors don’t demonstrate how the lab studies connect to the atmosphere but then produce a model parameterization. There are several steps between that must be first considered: (1) how common are bacteria in the atmosphere? (2) of these how frequent are IN-active species such as SNOWMAX? (3) how do the lab “single complexes” relate to atmospheric aerosols? Once these three are answered you might consider a cloud parameterization but, as it currently stands, none of the three are described in detail. This needs to be corrected if this “cloud parameterization” is to remain in the paper.

Other points:

CRITICAL : Previous study results should be contained for comparison in one or more figures. I note the data of Wood in Figure 8 but most obviously this should be done in Figure 3 regardless of if the size might be somewhat different. The reader needs to be allowed to compare to what is already known and what is new here. Ideally, data would also be shown in Figure 7 (i.e., in nucleation rate space).

Introduction (30): “. . .soot belong to these major constituents of ice crystal residues (e.g., Pratt et al. (2009); Kamphus et al. (2010); Twohy and Poellot (2005))” With the exception of a portion of the last reference these papers actually DON’T support soot as a major IN (mineral dust, yes). Please find other references although I think actual data supporting soot as an IN is rare outside a few lab studies.

Introduction (34): “Ice nucleating active (INA) bacteria, being ubiquitous in the atmosphere. . .” to my knowledge while INA bacteria have been FOUND in the atmosphere data do not indicate they are UBIQUIOUS. These are two VERY different terms.

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None of the references state they are ubiquitous, indeed the first reference (Moehler et al. 2008) cites a need for more studies in atmospheric abundance. This statement is not supported by the literature and needs to be toned down.

Within 4.2 Poisson distribution of identical IN (215): Unless I'm missing something it seems likely that the abundance of "complexes" relates to the concentration of SNOWMAX within the atomizer whereas this section discusses the relation to droplet volume. Further, as pointed out above, the reader is left unconvinced that complexes don't relate to the production method of SNOWMAX which could be subject to change from batch to batch. I think some statements here need to be made to make it clear the generation method and concentration of material is inherent in what is found about the distribution of IN. This should be explained in more detail. To be clear, as pointed out in line 233 the higher volume particles have more ice active material but the concentration chosen also determines this. Indeed it isn't until line 325 that the statement of 10^4 cells per droplet is made.

Summary (425): "We found that INA protein complexes controlling the ice nucleation behavior of *Pseudomonas syringae* bacteria belong to the most active IN considered up to now." More so than AgI? It would seem these complexes are often, if not always, somewhat less effective than some of the man-made cloud seeding agents. Specifically within LACIS or by all groups world-wide?

Statement on editing:

In general a very well written paper but will need to be edited for English. Numerous dropped punctuation marks and small grammatical errors.

Interactive comment on Atmos. Chem. Phys. Discuss., 12, 21321, 2012.

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