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Atmospheric Chemistry and Physics Discussions

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

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The comments below were written before I looked at the comments of the other reviewer who posted his/her remark before mine. Hence they are completely independent of any influence that the previous review could have had on my opinion.

GENERAL REMARKS

In this work the authors used the LACIS system to evaluate the immersion freezing behavior of the bacterium P. syringae in the form of the Snomax product. Compared to the immersion freezing test of larger droplets of more dense suspensions of Snomax, this is a relatively complicated technique (from the point of view of microbiologists who are well acquainted with the droplet test). However, I do not see the added value of the

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technique because the conclusions are essentially the same as those gained from the simpler technique in terms of the rates of efficiency of this INA bacterium. The authors claim that they are working with a small number of protein complexes per drop and that they can evaluate the number of protein complexes in each drop. One of the assumptions that they make for these calculations is that the absence of freezing means the absence of the protein complex. However, this has not been verified. Furthermore, individual protein complexes are not dispersed in the Snomax product. This product consists of whole bacterial cells that each harbor numerous protein complexes on their surfaces. It is possible to disperse the cells but I do not understand how the protein complexes were dispersed without breaking the cells and thus damaging the ice nucleation efficiency. It seems as if it would be more accurate to state that they can calculate the number of ice nuclei per drop. In this case, it is not clear what new information about ice nucleation activity is provided with this technique. The CHESS model could be useful for predicting the INA at a given temperature from data at other temperatures and hence could be used to parameterize models where the abundance of biological ice nuclei in the atmosphere is a parameter. However, they have not demonstrated the real predictive validity of this model for bacteria.

SPECIFIC REMARKS

Introduction

p 21323 l 16, This citation, Ward and DeMott, 1989, is not pertinent for the remark about the structure of the protein. These authors did not analyse protein structure.

p 21324, l 4: “since the seventies of the last century”, Change to “since the 1970’s”

p 21324, l 14: “bacteria related particles”, Change to “bacterial particles”

p 21324, l 14-20: The authors state that it is difficult to quantify and interpret the INA properties of polydisperse INA distributions. Why is this so? This statement should be justified.

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p 21324, l 20-23: When the authors mention “ice nucleation behavior” what are the parameters involved? They seem to indicate that threshold temperature is not part of nucleation behavior. It would be useful if the word “behavior” could be substituted with something more precise. Overall the authors seem to be saying that there are parameters of nucleation induced by INA proteins that have not yet been characterized (nucleation rate, for example). This paragraph belabors the point; they should just state that and explain why these parameters are important or more informative than threshold temperature.

p 21326, l 3: “consists of deadened”, change to “consists of non viable”

p 21356, l 6-7: “The Pseudomonas syringae bacteria used in Snomax production are grown”, change to “The strain of P. syringae used in Snomax production is grown”

Materials and Methods

p 21326, section on Snomax. The authors do not explain how the particles of different size ranges are generated and separated.

Results

p 21330, l 1-14: The authors argue that A and B class INA proteins are not present in detectable amounts among the 1000 to 10000 droplets tested. However, Figure 3 shows are small fraction of ice forming at temperatures warmer than -5°C and just a bit colder than -5°C. The rates are about 1/1000, so that would mean between 1 and 10 frozen droplets, assuming on average 1 particle per droplet. Why do the authors say that the class A and B nuclei are not detectable? Perhaps it would be better to say that they represent a small fraction of the total nuclei. I would like to remind the authors that this is the typical situation in INA bacteria, that the class C proteins are generally dominant.

p 21330, l 15-21: The increase of ice fractions with increasing particle size is not surprising. Furthermore, the fact that this increase is not linear with increasing particle

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size also seems to be intuitive. The protein that is responsible for INA of bacteria constitutes a small fraction of the total surface proteins of this bacterium. The cell size of P. syringae is about 1 μm. Furthermore, the cell membrane holds the INA protein structure to ensure its activity. Hence, very small particles are likely to not have any protein on them. Furthermore, if the particles represent cells that died and lysed during the industrial preparation of Snomax, then the protein configuration necessary for ice nucleation activity might have been lost. These particles would be expected to have lower activity or no activity relative to particles that arise from cells that fraction after lyophilization of the Snomax product. The authors could mention this either here or in the discussion to illustrate that their results are logical.

p 21330, l 24: The authors state that they can determine the number of protein complexes in a droplet. Do they, in fact, mean the number of ice nuclei? Do they assume that each protein complex leads to the formation of only 1 ice nucleus and that all protein complexes freeze under the temperatures tested here? Above, (p 21329, l 21-22) they indicate that the fact that droplets are unfrozen means that they do not contain even a single INA protein complex. But how do they know that? It is possible that certain INA protein complexes are formed in such a way that they are not efficient ice nucleators. I think that it would be advisable for the authors to modify their vocabulary to reflect what in fact they actually measured – ice nuclei. This remark means that the authors might not really know if they have monodisperse or polydisperse suspensions. If they are referring to particles, then yes they can verify that they have monodisperse suspensions, i.e. that the suspension contains single particles and of a single size. But if they are targeting monodisperse suspensions in terms of INA protein complexes, they do not know that this is true. They only know that they have single ice nuclei on average in a drop, and most of them active at about the same temperature (sharp increase in number of frozen drops and then saturation).

p 21335, l 17-19: The authors conclude that the INA protein complexes from P. syringae are the most active IN known. This information is surely not new and is well

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documented in the literature. The authors should indicate what new information they provide via the CHESS calculation that gives new insight into the nucleation capacity of P. syringae.

The application of the CHESS model to other systems is illustrated in FIGURE 8. The curves in this figure for the Maki et al data for P. syringae extrapolate based on a single data point. In one case this leads to predicting that there is ice nucleation activity above 0°C. There are many more data available in the literature on the INA of P. syringae that the authors could have chosen. Nevertheless, the model predicts what has already been observed experimentally for the ice nucleation spectra due to the protein of P. syringae as best illustrated by Orser et al, Figure 4, pg 363 (Orser, C., et al.: Cloning and expression of bacterial ice nucleation genes in Escherichia coli., J. Bacteriol., 184, 359-366., 1985.)

There are some English usage and punctuation errors. The authors should have the manuscript read by a physicist who uses English as a native language.

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