

Interactive comment on “Freezing nucleation apparatus puts new slant on study of biological ice nucleators in precipitation” by E. Stopelli et al.

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Received and published: 13 December 2013

Dear Prof. Vali, We appreciate you commenting on our manuscript. We agree that our method brings new possibilities for the elucidation of some aspects on heterogeneous ice nucleation. Still, it is just at its outset, so we are pleased to have the opportunity to discuss and to improve the possibilities for this research through the points emerging from your reply.

“Dorsey (1948) made effective use of this method”

The paper by Dorsey you referred to is rich in information. We are curious to go through its pages and discover what has been done previously and maybe to get some more ideas useful for our future research.

C3684

“The heading “effect of storage” doesn’t do justice to that. On the other hand, the interpretation offered for those changes requires more proof.”

The expression you used “evolution of a sample” is indeed a better description for the changes we observed during storage of samples. We will change the heading of that section in the final version of the paper. Regarding the results observed from the storage of samples at low temperatures, we are currently working to get more evidence on this aspect. First of all, we are trying to understand whether also different kinds of INA substances (soil, pure clay, fungal spores, snomax) show that behavior or if it is only living bacteria. If this is the case, then we could say it is part of a cellular activity.

“In Fig. 4, it would be useful to be able to trace which samples move up to higher temperatures.” “Did any of the samples from the -4 to -5 group move to colder temperatures?”

Specifically on Fig. 4 it is a nice possibility to underline which samples move up to higher temperatures and follow whether they move to colder temperatures over time. We are working on the improvement of this figure, to better underline the dynamics of tubes. Generally speaking, the two behaviors (decrease and increase of activity) are present at the same time, so some samples starting at -4°C moved down to -10°C and vice versa. The study of pure substances may help to clarify some aspects related to these different dynamics.

“The loss of activity after two dilutions in two of the samples and in two more after three dilutions is troubling. It isn’t easy to think of explanations for these losses and the authors didn’t offer one.”

The loss of INA during dilution and repeated freezing is in effect problematic. We are working to set up an efficient method for the optimization of progressive dilution steps. To date, we cannot provide a specific explanation, but we think that the loss may be due either to an incomplete recovery of all particles present in a tube or to the manipulating conditions destroying the ice nucleation active site. We could insert a consideration

C3685

about this point in the article.

Interactive comment on Atmos. Meas. Tech. Discuss., 6, 9163, 2013.

C3686