

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

**E. Stopelli et al.**

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Dear Dr Hill,

Thank you very much for the comments and suggestions expressed on our manuscript, they have been interesting to read.

“(Fig. 4, it looks to me like some tubes did contain INA bacteria initially and that they grew, probably at the expense of others killed by repeated freezing events; I think this is called cryptic growth).”

You suggested the data in Fig. 4 may indicate cryptic growth of INA bacteria. We are currently studying the behavior of different biological particles upon storage and

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freezing cycles. Still, to test the hypothesis that INA bacteria can grow by benefiting from freezing damage to other cells, a mixture of different microorganisms should be tested as well.

“So I think you should promote the utility of the machine for measuring IN right down to  $-25^{\circ}\text{C}$ .” “References good, but with regard to other people also currently developing new ways to measure immersion freezing don’t forget Iannone et al 2011 in ACP, 11, p 1191.”

Thanks for the description of the method you are currently employing to assess freezing events. Unfortunately, as mentioned in the paper (line 19 page 6), we cannot go down to  $-25^{\circ}\text{C}$  with our apparatus, because background effects (distilled water, surface of tubes) provoke freezing in blank samples (not containing IN active material) starting from  $-15^{\circ}\text{C}$ . There is a trade-off between decreasing the detection limit through a bigger drop volume and simultaneously increasing background counts. Although we theoretically could dilute a sample almost infinitely with distilled and filtered water to determine the much larger concentrations typically found at  $-25^{\circ}\text{C}$ , we would increase the background count because of our water not being absolutely free from IN active at those temperatures. For freezing nucleation tests at colder temperatures we think there are better suited instruments, such as the one described by Iannone et al (2011) in the paper you recommended to us (and which we will refer to in a revised version of the paper). It uses drops with diameters around 0.1–0.3 mm, producing respectively a 75'000–3'000 times smaller volume than the smallest drops we can analyse (40 microliters).

“With regard to the experiment underlying Table 2, it’s a good idea, yes, and one we’ve been exploring as well but it’s not reliant upon having a LINDA device. However, the LINDA device did uniquely permit you to detect the shifts in freezing temperatures upon repeated freeze-thaw events in Table 2. Maybe that aspect should be foregrounded more”

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Thank you for your suggestion to stress the point that the difference that LINDA makes to the progressive isolation of ice nucleators lies in the possibility to follow small shifts in freezing temperatures. We are currently planning a set of tests for the improvement of the quality of this isolation procedure.

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