Biogeosciences Discussions

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Comment on bg-2020-474

Anonymous Referee #2

Referee comment on "Ice nucleation by viruses and their potential for cloud glaciation" by Michael P. Adams et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2020-474-RC2, 2021

This manuscript presents results on the ice nucleation activity (INA) of viruses. Viruses were not known to possess INA, so this manuscript reports novel and very interesting experiments on viral INA tested by immersion freezing over a range of temperatures between -15 and -35 degrees C. As the abundance of virus particles in the atmosphere is considered to be very high, their INA activity may be significant in climate processes. Therefore this manuscript falls within the scope of the journal.

I find these results interesting but not totally convincing.

The authors were careful to include important controls in their experiments: in addition to negative controls consisting of the buffer used for viral suspensions, controls included INA tests of the virus host strains, which is necessary to show that no carryover of bacterial INA was present in virus suspensions. The bacterial and archaeal host strains indeed did not possess any significant INA. Moreover, the authors examined the virus genomes and proteins for structural similarities of viral proteins to known bacterial proteins with INA. They found very little similarity.

However, an essential control to show no carryover of any bacterial INA into virus suspensions was not included in the experiments: an “extraction” control consisting of uninfected bacterial host cells going through the same procedure for extracting viruses. For example, this negative control would undergo the same centrifugation steps as for virus purification, and a sample of the gradient at the same depth as the virus band should be taken. Here is why I consider this essential:

All research on ice nucleation activity of bacteria was done at temperatures between -10 and 0 degrees centigrade. At this range, only intact bacterial cells are INA; purified bacterial INA proteins are significantly less active (at lower temperatures, attributed to the stabilization effect of the bacterial outer membrane). Other cell components after cell lysis are not active at these temperatures. The authors did their experiments of virus INA at significantly lower temperatures, from -15 to -35 degrees C. Indeed, at these temperatures the freezing temperature of most viruses tested is higher than the freezing temperature of buffer and of intact host cells. However, we do not know if bacterial cell lysates produced during virus extraction procedures are ice nucleation active at these temperatures. All we know from previous research of INA bacteria is that bacterial cell

lysates are inactive at temperatures above -10 degrees C; we know nothing about their INA at -15 degrees and below. So we cannot rule out that the observed INA of viruses is in fact the activity of bacterial cell components carried over to virus suspensions. The authors partially addressed this concern in the experiment presented in Figure S3: a second purification of one virus preparation did not alter the INA activity of the virus. Did this additional purification eliminate any carryover of host cell components into the virus preparation? The conclusion would be more concrete if this was done for all viruses tested. An "extraction" control would clarify this.

Specific comments on the manuscript:

Page 2, line 52: please include the original reference on bacterial ice nucleation from S.E. Lindow et al (LINDOW, S.E.;ARNY, D.C.;UPPER, C.D.;BARCHET, W.R. The role of bacterial ice nuclei in frost injury to sensitive plants. In: Li, P.H.; Sakai, A. (eds.). Plant cold hardiness and freezing stress: Mechanisms and crop implications. Academic Press. New York. 1978. p. 249-263.).

Page 5, Section 2.1 of Methods: The suspension of archaeal virus particles was done in K-phosphate buffer with NaCl. This information is missing from this section and the reader does not understand the use of a NaCl negative control in INA experiments in section 2.3 (line 204) later in the manuscript.

Page 6, line 156 and Figure S2: Are these viral protein profiles in agreement with reports in literature? Do they confirm the absence of non-viral proteins originating from host bacteria?

Page 7, Section 2.3: Please provide more details about the experimental procedure: mention the range of temperatures used during the ice nucleation experiments; how many droplets from each sample were tested; what was the rate of temperature drop during the tests.

Page 8, line235: why the most ice nucleation active virus Phi12 (shown in Fig. 1E, page 21) were not included in the study of the sub-viral particles' INA?

Page 9, lines 246-247: please rephrase this sentence with respect to syntax. Excessive use of word "hence".

Page 9, section 3.2 title: modify to "Genetic analysis of ice nucleation active virus particles".

Page 9, line 273: Please rephrase: "...the source of INA might also be of proteinaceous origin".

Page 10, line 274: Please rephrase: "virus particles possessing similar structure and function to known bacterial ice nucleation proteins as explanation….".

Page 10, lines 275-276: Please improve this sentence.

Page 11, lines 305-316: Please discuss whether the reported marine organics and sea spray aerosols are chemically or physically related somehow to viruses (information from the 3 references mentioned in this paragraph).

Figures S5 and S7: the archaeal hosts have some INA too, higher than the buffer and close to or same as the respective viruses. This was not the case with the bacterial hosts: they all had the same INA as the buffer controls. Therefore the conclusion that the INA of the viruses in these figures is not some "contamination" effect by the host is not totally

supported.