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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Activation of Intact Bacteria and Bacterial Fragments Mixed with Agar as Cloud Droplets and Ice Crystals in Cloud Chamber Experiments" *by* Kaitlyn J. Suski et al.

Anonymous Referee #2

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Suski et al. present experimental data from three cloud chamber expansions at AIDA where a suspension of bacterial particles and their fragments mixed with agar were injected into the chamber. The goal appears to be to understand and contrast the role of intact bacterial cells versus bacterial fragments mixed with agar in the nucleation of cloud droplets than can then undergo immersion freezing. This is certainly a relevant question to the atmospheric science community, though the presence of agar makes the results more relevant to interpreting past and future laboratory studies that use nebulized suspensions of bacterial since agar is not an atmospherically relevant particle component. In the end few new findings are really presented from these experiments, and the significance and originality of this work is rather low as a result. Perhaps the

most novel aspect is the use of a cloud expansion chamber. I also found the paper hard to follow, and do not think the very narrative style of discussing each of the three cloud expansions to be an effective way to communicate, analyze, and synthesize the results. The main finding is that intact bacterial particles make a small contribution to CCN activation and thus cloud droplets, and thus also a small contribution to immersion freezing and ice crystal production. This is certainly a worthwhile finding and it should really be made the focus of this paper, but it also requires better support from the available data. I was not very convinced by the interpretation of these results, especially since the intact bacterial were such a small contribution to the total particle numbers to begin with. The single-particle mass spectrometer SPLAT is used to determine the chemical composition as a function of size. This analysis is hampered by the lack of significantly distinct mass spectral features that can be used to reliably distinguish the fragment+agar particles from the intact bacteria. As it is a single-particle instrument, as droplet and ice crystal activation occurs on individual particles, I found it odd that average mass spectra were presented, as opposed to trying to determine the fraction of each type of particle as a function of particle size. In summary, while the topic is of interest, not much new insight is presented here, and the presentation and discussion is quite unclear and hard to follow. The main singular conclusion that intact bacteria do not activate as CCN or into ice crystals needs further support. Extensive revisions are required to achieve this, and publication in ACP may not be warranted unless these major issues can be properly resolved.

I find the narrative style of describing each expansion experimental chronologically to be an ineffective way to communicate the results. At the least the important characteristics of each expansion and how they differ from each other must be discussed. E.g. how do the aerosol concentrations and size distributions differ? How do the thermodynamic conditions of the expansion differ? It looks like expansion 1 reaches a higher supersaturation of at least 102% RHw. Stating the maximum SS/RHw reached in each expansion is critical for understanding the CCN activation, just as stating the maximum RHi and minimum temperature reached is critical for understanding the ice

crystal production.

In describing each expansion the authors mostly state what data is plotted in the various figures, as opposed to actually discussing, interpreting, and synthesizing the results. As written the description of the expansion experiments is not very meaningful.

On “hydrophobic” intact bacterial particles. So long as the particle surface is wettable these large particles will still activate under the high supersaturations reached here. It appears that RHw usually hits 101%, so 1% SS. This is why it is important to state the maximum RHw. At high a high SS large particles with a very small hygroscopicity of kappa $\sim$ 0 will still activate into droplets.

What would really help this analysis is if the /fraction/ of the two type types/size modes of particles that activate into cloud droplets and ice crystals in each expansion could be estimated. This should be possible from the data. It is rather misleading to say that the intact bacteria make a small contribution to cloud droplets and ice crystals considering they are a small fraction of the initial particles to begin with. What is needed are the particle fractions, ie the CCN/CN and INP/CN ratios. This will also make these results more useful in a quantitative manner for other researchers. As presented the results reported here cannot be used in a meaningful to for example describe the CCN or IN properties of these particles types in a model.

Pg 1/line 30: Please provide several references for this b/g info on the role of bacteria in the atmosphere and clouds. “Murray, 2012 and references therein” is not sufficient. One suggestion:

DeMott, P. J. and Prenni, A. J.: New Directions: Need for defining the numbers and sources of biological aerosols acting as ice nuclei, Atmos. Environ., 44(15), 1944–1945, doi:10.1016/j.atmosenv.2010.02.032, 2010.

2/7: Contact angle would explain only the wettablilty, not hygroscopicity, of bacteria. Hygroscopicity really refers to the ability of a dissolved solute solution to take up water.

2/15: The introduction is quite spare on references to the many prior papers on the ice nucleation properties of bacteria/Pseudomonas syringae/Snomax. Some of these get mentioned much later in the paper but they also belong in the introduction, or else it appears that the authors are not familiar enough with the topic under study here. Some suggestions, but there are many more:

Pandey, R., Usui, K., Livingstone, R. A., Fischer, S. A., Pfaendtner, J., Backus, E. H. G., Nagata, Y., Fro hlich-Nowoisky, J., Schmu ser, L., Mauri, S., Scheel, J. F., Knopf, D. A., Po schl, U., Bonn, M. and Weidner, T.: Ice-nucleating bacteria control the order and dynamics of interfacial water, Sci. Adv., 2(4), e1501630–e1501630, doi:10.1126/sciadv.1501630, 2016.

Lindow, S. E., Arny, D. C. and Upper, C. D.: Bacterial Ice Nucleation: A Factor in Frost Injury to Plants, PLANT Physiol., 70(4), 1084–1089, doi:10.1104/pp.70.4.1084, 1982.

Després, V., Huffman, J. A., Burrows, S. M., Hoose, C., Safatov, A., Buryak, G., Fröhlich-Nowoisky, J., Elbert, W., Andreae, M., Pöschl, U. and Jaenicke, R.: Primary biological aerosol particles in the atmosphere: a review, Tellus B Chem. Phys. Meteorol., 64(1), 15598, doi:10.3402/tellusb.v64i0.15598, 2012.

Polen, M., Lawlis, E. and Sullivan, R. C.: The unstable ice nucleation properties of Snomax® bacterial particles, J. Geophys. Res. Atmos., 121(19), 11,666-11,678, doi:10.1002/2016JD025251, 2016.

Wex, H., Augustin-Bauditz, S., Boose, Y., Budke, C., Curtius, J., Diehl, K., Dreyer, A., Frank, F., Hartmann, S., Hiranuma, N., Jantsch, E., Kanji, Z. A., Kiselev, A., Koop, T., Möhler, O., Niedermeier, D., Nillius, B., Rösch, M., Rose, D., Schmidt, C., Steinke, I. and Stratmann, F.: Intercomparing different devices for the investigation of ice nucleating particles using Snomax® as test substance, Atmos. Chem. Phys., 15(3), 1463–1485, doi:10.5194/acp-15-1463-2015, 2015.

Pummer, B. G., Bauer, H., Bernardi, J., Bleicher, S. and Grothe, H.: Suspendable

macromolecules are responsible for ice nucleation activity of birch and conifer pollen, Atmos. Chem. Phys., 12(5), 2541–2550, doi:10.5194/acp-12-2541-2012, 2012.

Turner, M. A., Arellano, F. and Kozloff, L. M.: Components of ice nucleation structures of bacteria, J. Bacteriol., 173(20), 6515–6527, 1991.

Turner, M. A., Arellano, F. and Kozloff, L. M.: Three separate classes of bacterial ice nucleation structures, J. Bacteriol., 172(5), 2521–2526, 1990.

Attard, E., Yang, H., Delort, A.-M., Amato, P., Pöschl, U., Glaux, C., Koop, T. and Morris, C. E.: Effects of atmospheric conditions on ice nucleation activity of <i>Pseudomonas</i>, Atmos. Chem. Phys., 12(22), 10667–10677, doi:10.5194/acp-12-10667-2012, 2012.

Hartmann, S., Augustin, S., Clauss, T., Voigtländer, J., Niedermeier, D., Wex, H. and Stratmann, F.: Immersion freezing of ice nucleating active protein complexes, Atmos. Chem. Phys. Discuss., 12(8), 21321–21353, doi:10.5194/acpd-12-21321-2012, 2012.

Vali, G., Christensen, M., Fresh, R. W., Galyan, E. L., Maki, L. R. and Schnell, R. C.: Biogenic ice nuclei 2. Bacterial sources, J. Atmos. Sci., 33(8), 1565–1570, 1976.

2/23: Macromolecules of protein aggregates are known ice nucleants produced by bacteria. Are the macromolecules really a “solid nucleus”. This definition doesn’t align with the role of macromolecules as ice nucleants. 2/26: Consider using “macromolecules” instead of “nano-INP”, as this is the terminology more widely used.

3/3: There is also evidence of biological ice nucleating macromolecules attaching to particles such as dust, and also evidence for mixed “dust-bio” particles, such as from the first author’s prior work. This would also seem to motivate this study and should be discussed.

O'Sullivan, D., Murray, B. J., Ross, J. F. and Webb, M. E.: The adsorption of fungal ice-nucleating proteins on mineral dusts: a terrestrial reservoir of atmospheric ice-nucleating particles, Atmos. Chem. Phys., 16(12), 7879–7887, doi:10.5194/acp-

16-7879-2016, 2016.

3/27: It is a bit confusing that the stated purpose of FIN-1 is to intercompare SP-MS instruments and yet that is not done here. Please explain more to better put this particular study in the context of FIN-1.

4/14: Usually the particle density and shape are varied to arrive at a good overlap in the SMPS and APS size distributions, instead of just assuming values. Also what are these assumed values based on? Isn’t the SPLAT instrument a great way to actually measure the shape factor and density of these particles?

Khlystov, A., Stanier, C. and Pandis, S. N.: An Algorithm for Combining Electrical Mobility and Aerodynamic Size Distributions Data when Measuring Ambient Aerosol, Aerosol Sci. Technol., 38(sup1), 229–238, doi:10.1080/02786820390229543, 2004.

Beddows, D. C. S., Dall’osto, M. and Harrison, R. M.: An Enhanced Procedure for the Merging of Atmospheric Particle Size Distribution Data Measured Using Electrical Mobility and Time-of-Flight Analysers, Aerosol Sci. Technol., 44(11), 930–938, doi:10.1080/02786826.2010.502159, 2010.

4/27: Please state the cut-size of these two CVIs.

5/10: Why were these two cultures chosen? Much existing work of course on P. syringae, but what about PF CGina 01? Also unclear if these are both used in all three expansions?

6/15: Do you determine which particles are intact bacteria simply based on a size threshold, and if so what is it and what is it based on?

7/6: Qualitative terms such as “very high CCN activation efficiency” are often used. The hygroscopicity of the different aerosol types should be estimated from the maximum supersaturation observed, and the size distribution.

7/14: Why would these large bacterial particle not activate? Again need to know the

maximum in the RHw. Have you tested that these large particles once activated into cloud droplets survive the PCVI? I agree with the other referee's comments regarding the bacterial possibly rupturing during cloud droplet activation and certainly during freezing. Showing the average mass spectra is not that meaningful here. The small but important number fraction of intact bacteria will be obscured by averaging. An estimate of the number fraction of intact bacteria vs. fragments+agar in the different size modes would be really useful.

9/14: Again, need to know what the CCN/CN fraction was at what max RHw to really evaluate the hygroscopicity of the particles. "very high CCN activation efficiency" is not quantitative.

Expansion 3 seems unique in that there is much more ice produced even though the aerosol seems similar to the other expansions, but the reason for this difference is not discussed.

10/14: "Nevertheless, the data presented here show that bacterial fragments mixed with agar preferentially activate as droplets and are the only particles observed in ice residuals." This is essentially the singular conclusion reached from this study, and it is an interesting one. I strongly suggest making this aspect the focus and providing more data and analysis that better supports this conclusion.

Fig. 2 and other mass spectra: The two types of particles appear to have no unique ion markers, just differences in the relative ion signals. This makes separating the two particle types out based on their mass spectra rather challenging.

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