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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.

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Response to Anonymous Referee #2 Thank you for your comments and suggestions. We have addressed them in the revised version of the paper. Below are our responses to your comments.

-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. 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.

The reviewer clearly came to the conclusion that the paper presents data which indicates that particles composed of bacterial fragments mixed with agar activated to form either droplets or ice crystals much more efficiently than intact bacteria. As the referee notes, this is an important finding. Agar or other growth media is present in all laboratory studies that are done with cultured bacteria and the present study shows that it plays an important role that needs to be considered when interpreting CCN and IN activation data. To the best of our knowledge, we present the 1st measurements of both size distributions and composition of cloud residuals of these bacteria, which made it possible to identify which particles were preferentially activated. As we note in the paper, not knowing which particles were activated led some previous studies to calculate the fractions of particles that activate as droplets and ice crystals relative to the number of intact bacteria only or to the total number of particles in the chamber. Each of these choices led to INP/CN values that do not accurately describe the activity of either particle type. Another important new finding in this study is that the relative fraction of agar in these particles gradually decreases with particle size, which affects their hygroscopicity and hence CCN and IN activity.

-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.

We have revised the paper to improve the flow, better illustrate the key points, and address reviewer's concerns. We also have added Table S1 that lists experimental parameters for the 3 expansions, including the CCN/CN and INP/CN ratios.

-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.

It is important to keep in mind that in most previous laboratory studies of aerosolized cultivated bacteria the fraction of intact bacteria was small. Our paper is the first study that shows which of the particle types were activated as CCN and INP more efficiently when both intact bacteria and fragments mixed with agar are present at the same time. Aerosolized intact bacteria and bacterial fragments mixed with agar are composed of the same compounds, albeit present in different ratios. Therefore, it is not surprising that individual mass spectra of all particles in the chamber have the same mass spectral peaks, as shown in the new Figure 3, just with different relative intensities. All of the bacteria and bacterial fragment particles, however, contain distinct phosphorus-containing peaks that separate them from pure agar. Additionally, we demonstrate for the first time that the relative fractions of agar and bacterial fragments are changing with particle size, thereby, effecting their mass spectra. Figure 12 of the revised manuscript and Figure 3 in the response to reviewers clearly illustrate this point. As a result, distinguishing between intact bacteria and large bacteria fragments mixed with agar based on their mass spectra alone is impossible. However, a clear distinction

between these two particle types is clearly seen in the particle size distributions that show two distinct peaks, one at $\sim$180 nm and the other at $\sim$850 nm (dva). Simultaneous measurements of single particle composition and size provides the means to compare CCN and IN efficiency of two particle types. The fact that there were more particles composed of bacterial fragments mixed agar than intact bacteria does not invalidate the results. An examination of the size distributions shows that the vast majority of particles larger than 600 nm (dva) are intact bacteria. Inspection of the dva size distributions of activated particles, which does not show the distinct peak that corresponds to intact bacteria, clearly indicates that under the experimental conditions in the AIDA chamber, in which not all particles activate as cloud droplets (Table S1), the more hygroscopic smaller particle mode, composed of bacterial fragments mixed with agar, activate with significantly higher probability than intact bacteria.

-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.

We chose to present three separate experiments conducted on two bacteria to demonstrate that the data, and hence the conclusions, are perfectly reproducible. Nevertheless, we have revised the paper to improve the flow and better illustrate the key findings. We agree that given the importance of this topic the findings, which were reported here for the first time, call for follow-up studies.

-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 addition to presenting all of the RH data in the figures, we have added a table to the supplemental section (Table S1) that includes all of the expansion details. We chose this narrative style to emphasize the fact that the results of these three separate experiments, on two different bacteria, are perfectly reproducible. The differences between the thermodynamic conditions of the three expansions were minor. We agree with the reviewer that the issue of the precise value of SSw/RHw is central to most CCN and IN activation studies from which parameters for models are being calculated. This, however, is not the goal of the present study. Below, we will return to the discussion of RHw measurements. -We note that the reviewer concludes the review by stating (copied from below): "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.

We agree with this conclusion. We have also revised the manuscript and added the additional data you requested.

-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.

We have revised the discussion to more clearly convey our interpretations of the results.

-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 âĹij 0 will still activate into droplets.

The revised plots show the raw 1-sec data from tunable diode laser (TDL), which was used for in-situ measurements of the water vapor concentrations using absorption spectroscopy throughout the expansions. As discussed in detail elsewhere, (e.g. Fahey et al. (2014; www.atmos-meas-tech.net/7/3177/2014/); Hiranuma et al., 2014a (www.atmos-chem-phys.net/14/13145/2014/)) the accuracy of the measured relative humidity with respect to water (RHw) and ice (RHi) is $\pm$ 5 %. In the three expansions presented here the maximum values of the measured RHw were 97% for expansion 1, and 96% for the other two (Table S1). Given that cloud droplets were clearly formed, the values of RHw in the original plots were scaled. The high uncertainties in the RH measurements make it impossible to precisely determine SSw, which is necessary to calculate particle hygroscopicity parameters; however, this was not the goal of the present study. Again, the questions at hand are, which of the two particle types are more CCN and IN active under the same conditions, and does agar play an important role? The CCN/CN ratios listed in Table S1 indicate that not all particles in the chamber activated as cloud droplets, providing a possible explanation for why the more hygroscopic bacterial fragments mixed with agar activate, while the less hygroscopic intact bacteria do not.

-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.

We calculated the fractions of particles that activated into cloud droplets and ice crystals in all three expansions and provided this information in Table S1 and in the text. Since it is impossible to distinguish between intact bacteria and large bacteria fragments mixed with agar, we cannot calculate the fraction of intact bacteria that act as CCN and INP based on MS alone. However, the size distributions of cloud residuals, shown in Figures 5, 10 and 14, exhibit no distinct peak for intact bacteria and are

dominated by smaller particles composed of bacterial fragments mixed with agar.

-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.

The fractions of the intact bacteria in these experiments are rather typical for laboratory studies on cultivated bacteria (e.g. Wex et al., 2015;Wolf et al., 2015;Möhler et al., 2008). If intact bacteria were, as typically assumed, more active than the bacterial fragments mixed with agar, they would be relatively enhanced in cloud residuals. Such enhancement would yield a more prominent peak at $\sim$850 nm in the dva size distributions, as compared to the peak observed for the particles before the expansions. We clarified the text to say that relative to the bacterial fragments mixed with agar the fraction of intact bacteria, if present in activated particles, is much smaller than that in the original sample. 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., Rosch, M., Rose, D., Schmidt, C., Steinke, I., and Stratmann, F.: Intercomparing different devices for the investigation of ice nucleating particles using Snomax (R) as test substance, Atmos Chem Phys, 15, 1463-1485, 10.5194/acp-15-1463-2015, 2015.

Wolf, R., Slowik, J. G., Schaupp, C., Amato, P., Saathoff, H., Möhler, O., Prevot, A. S. H., and Baltensperger, U.: Characterization of ice-nucleating bacteria using on-line electron impact ionization aerosol mass spectrometry, J Mass Spectrom, 50, 662-671, 10.1002/jms.3573, 2015.

Möhler, O., Georgakopoulos, D. G., Morris, C. E., Benz, S., Ebert, V., Hunsmann, S., Saathoff, H., Schnaiter, M., and Wagner, R.: Heterogeneous ice nucleation activity of bacteria: new laboratory experiments at simulated cloud conditions, Biogeosciences, 5, 1425-1435, 2008.

-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.

These have been added to the paper and listed in Table S1.

-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.

The goal of this manuscript is not to calculate the CCN and IN fractions to be used in a model. As we discuss in the paper, the size, number concentration, and relative fraction of the small particles containing bacterial fragments mixed with agar are affected by the solution/suspension concentrations of agar, intact bacteria, and cell fragments, how old it is, as well as how the bacteria culture was grown and prepared. Future studies need to be aware of the unavoidable presence of agar or other growth media that can greatly change the results. We cannot overemphasize that in order to use laboratory studies to generate useful parameters for atmospheric models one must make sure to eliminate artifacts that can affect CCN and IN activity. In the case of cultured bacteria, agar or other growth media is present in all laboratory studies and its effect makes it impossible to extrapolate the data to atmospheric conditions, where no agar is expected.

-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.

References have been added.

-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.

To the best of our knowledge there are no papers on the hygroscopicity of intact bacteria. The contact angle measurements were used to infer hygroscopicity in the referenced study.

-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., Frohlich-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.

Some of these papers were already in the introduction, but we have added the suggested references that were not there and expanded the introduction.

-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.

This has been revised to read, “In immersion freezing, the INP first activates as a liquid cloud droplet, when the RH over water (RHw) exceeds 100% RH, and subsequently ice forms, which means that this ice formation mechanism is tightly connected to the particle CCN activity.”

-2/26: Consider using “macromolecules” instead of “nano-INP”, as this is the terminology more widely used.

It has been changed to macromolecules.

-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. 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/acp16-7879-2016, 2016.

This reference has been added and this point added to the introduction.

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

This discussion has been expanded. It now reads: "The Fifth International Ice Nucleation Workshop (FIN) was a three-part study that aimed to compare a number of single particle mass spectrometers and ice nucleation instruments. The data presented here were collected during FIN-1, which took place in November of 2014 at the AIDA cloud chamber at KIT in Karlsruhe, Germany. Ten single particle mass spectrometers from several research groups around the world were brought together for FIN-1 to simultaneously characterize the size and composition of various types of aerosol particles, including those being used in the AIDA chamber to study their activity as CCN and INPs, Various aerosol types were sampled from the AIDA cloud chamber before, during, and after expansions forming cloud particles and each of these mass spectrometers sampled using their own protocol. The results of the intercomparison will be the subject of future publication. The present manuscript presents the results of measurements, made by miniSPLAT only, during three expansions using two bacteria. "

It is important to point out that the extremely high detection efficiency and sensitivity of our SPMS, including its unmatched sensitivity to small particles, as well as its dual data acquisition mode, (Zelenyuk et al. 2015) makes it uniquely suitable to characterize both types of particles before, after, and during the expansions with high temporal resolution. Zelenyuk, A., Imre, D., Wilson, J. et al. J. Am. Soc. Mass Spectrom. (2015) 26: 257. https://doi.org/10.1007/s13361-014-1043-4

-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?

The values are not assumed. The effective density of the bacteria of 1.4 g cm-3 has been previously measured and presented in the paper referenced below. It was used here to achieve a good overlap of the SMPS and APS size distributions, assuming particle sphericity. We have added more clarification on this in the text. Moreover, this value is in a good agreement with effective density of 1.38 g cm-3 measured by miniSPLAT for intact pseudomonas syringae during the FIN-1 campaign.

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., Rosch, M., Rose, D., Schmidt, C., Steinke, I., and Stratmann, F.: Intercomparing different devices for the investigation of ice nucleating particles using Snomax (R) as test substance, Atmos Chem Phys, 15, 1463-1485, 10.5194/acp-15-1463-2015, 2015. 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.

This information has been added to the paper (Table S1 and Figures 4, 9, and 13).

-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?

Pseudomonas syringae and PF CGina are two different strains of Pseudomonas bac-

teria. PF CGina has been studied previously and has been shown to be an efficient INP at modestly supercooled temperatures. It has also been observed in glacier meltwater. More information about PF CGina has been added to the introduction to address this issue. Pseudomonas syringae was used in Expansion 1, while PF CGina was used in Expansions 2 and 3. The data presented in the paper indicate that the findings are consistent for both bacteria strains.

-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?

Yes, this is explained in the text. The particles in the AIDA chamber have 2 distinct size modes, with the larger size mode (dva above $\sim$700 nm) corresponding to intact bacteria.

-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.

We have calculated the CCN/CN and have added it to the paper.

-7/14: Why would these large bacterial particle not activate? Again need to know the maximum in the RHw.

As the reviewer notes, the data presented here indicate that under these experimental conditions the large intact bacteria particles are ineffective CCN, which, in essence, implies that they are weakly hygroscopic. In contrast, smaller particles contain INactive bacterial fragments and hygroscopic agar. Less than half of the particles in the AIDA chamber activated to form cloud droplets, thus there was competition for water vapor during the expansion or the SSw did not reach a high enough value to activate the less hygroscopic particles in the chamber. Here we are being asked to speculate as to why intact bacteria are less hygroscopic, which is beyond the scope of the present paper. We note that the interaction between water and live cells/bacteria

and the mechanisms by which they prevent water from entering into the cell are the subject of many research papers. The issue of the large uncertainties in the measured RHw in the AIDA chamber have been already discussed above.

-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.

The transmission efficiencies of droplets of this size have been characterized and are presented in Hiranuma et al., 2014a (www.atmos-chem-phys.net/14/13145/2014/). However, bacteria bursting in the PCVI has not been tested directly in this study, but it is not clear what the mechanism for not surviving the PCVI would be. The data do not support the idea that intact bacteria burst upon drying in the PCVI: (a) The bacteria do not all burst upon drying, as they were all dried prior to injection into the chamber and we still see intact bacteria in the chamber; (b) If they burst in the PCVI, we would expect to see a large increase in the number of particles measured by the CPC downstream of the PCVI, which was not observed; (c) Intact bacteria do not all burst upon activation or freezing because after the expansion there are still intact bacteria present in the cloud chamber. In addition, the ratio of the two particle types remain constant in the size distributions before and after the expansion, as shown in Figure 7. Therefore, we conclude that the evidence suggests that the idea of bursting intact bacteria does explain the fact that intact bacteria are not present in cloud residuals.

-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.

As we already discussed above, it is not surprising that individual mass spectra of all particles in the chamber have nearly the same mass spectral peaks, as shown in the new Figure 3, albeit with different relative intensities. Both aerosolized intact bacteria and bacteria fragments mixed with agar are composed of similar compounds

mixed at different ratios. We have found that the relative fractions of agar and bacterial fragments change with particle size, effecting their mass spectra (Figure 12 of the revised manuscript and the figure above). The distinction between the two particle types is based on simultaneous measurements of single particle composition and size. As we noted above, particles composed of bacterial fragments mixed with agar that are larger than 700 nm have the same dva and indistinguishable MS from intact bacteria. However, the dva distribution of the intact bacteria has a distinct peak at $\sim$850 nm, while the dva distribution of bacterial fragments mixed with agar does not have a peak in this region.

-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.

We already discussed the dependence of particle mass spectra on particle size. Moreover, if intact bacteria were more active than the fragments, they would be enhanced in cloud residuals, resulting in a more prominent larger particle mode in dva size distributions as compared to the clear peak of the intact bacteria mode detected before the expansions.

-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.

We have provided the calculated CCN/CN fractions in the revised text and in Table S1. We discussed the issue of RHw above.

-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.

Actually, during Expansion 3 the concentration of ice crystals is lower as described in the text. More ice crystal residuals were sampled and characterized during Expansion 3 because instead of the PCVI, the IS-PCVI was used to select larger cloud particles as described in the text and shown in Figure 13.

-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.

We agree, the focus of the paper was to determine which of the two particle types was more CCN and IN active. We have revised the discussion to make that more clear. We have also added the additional data you requested. It is worth noting that in addition to this main point, this manuscript demonstrates the importance of agar on cloud nucleation in laboratory studies, and presents the new finding that particle composition strongly depends on size.

Interactive comment on Atmos. Chem. Phys. Discuss., https://doi.org/10.5194/acp-2018-594, 2018.

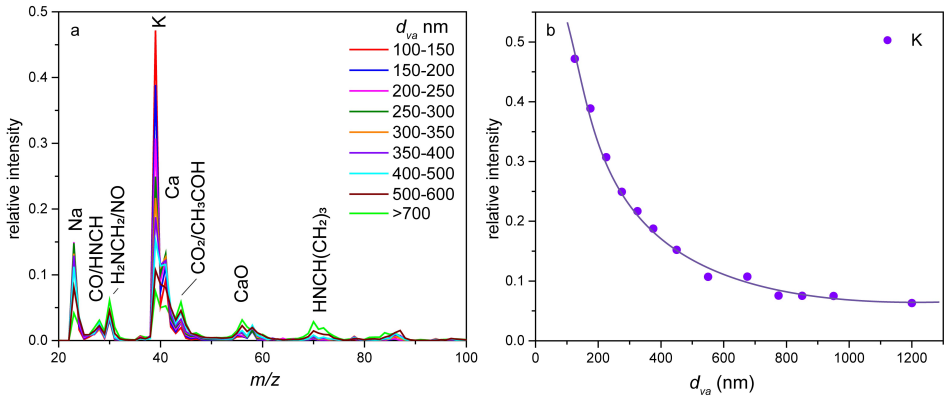

Fig. 1. Response Figure 3