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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Survival and ice nucleation activity of bacteria as aerosols in a cloud simulation chamber" *by* P. Amato et al.

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We thank Referee 2 for raising weaknesses in the discussion manuscript. Our responses to each comment are indicated below:

Referee#2: Comments: Page 4059; line 21 – define AIDA right after "AIDA".

Response of the authors: This has been corrected in the revised manuscript.

Referee#2: Page 4060; Recommend authors to include an introduction to their experimental approach after introducing the AIDA chamber and to direct the reader to Table 1. It could be as simple as a very brief overview of their experiment types (c, d, and e markers listed in Table 1). Suggest authors rearrange section to include chamber

experiment descriptions follow by sampling frequency and SMPS/APS descriptions.

Response of the authors: Section 2.1 was reorganized following Reviewer 2's recommendations.

Referee#2: Page 4060; line 8- "For the ageing experiments at constant atmospheric pressure" – what does this mean? Is the aerosolization process not the same for all experiments? Where there experiments for which this spraying/evaporating was not true?

Response of the authors: the aerosolization process was similar for all the experiments, so the specification that this was "for the ageing experiments at constant atmospheric pressure" has been removed.

Also, could the authors provide a discussion on how the aerosolization process used in this study relates to any hypothesized natural aerosolization processes? Bacteria can be emitted from spray (ocean) but also is often dry generated. The comparison is later made to bacteria released from agricultural fields, yet agricultural emissions are not necessarily release from a spray.

Response of the authors: It is true that spraying cultivated bacteria in a simulation chamber is probably different from naturally grown bacteria aerosolized by wind or bubble bursting. A paragraph discussing the different ways of aerosolization was inserted in the text, Section 3.3 (lines 323-338 of the revised manuscript): "Aerosolization, i.e. the transfer of cells from a solid surface or from a liquid to the air, is a critical step. In nature, the dragging forces created by wind on surfaces generate aerosols by saltation/blasting phenomena (Grini et al., 2002) and result in increased amounts of airborne microorganisms during high wind speed events (e.g., Lindemann and Upper, 1985). Splashing raindrops on surfaces colonized by microorganisms like plant leaves also lead to the aerosolization of living bacteria (Graham et al., 1977). From liquids, a well-know process of aerosolization is bubble-bursting (Blanchard and Syzdek, 1982). This is actually a phenomenon by which certain types of cells in a community are pref-

erentially aerosolized, thus adding a new layer of complexity in the process of bacterial aerosolization as it results in dissimilarities between the microbial composition in the bulk liquid source and in the air above (Agogué et al., 2005; Fahlgren et al., 2015). The complexity of this phenomenon was probably not reflected in our experimental setup, with bacterial cells being sprayed from liquid suspensions. However, the results presented here only considered bacteria already aerosolized and avoided taking into account the aerosolization step. Hence, considering that the process of aerosolization did not affect subsequent survival rates as aerosol, we can place our results in natural atmospheric context."

Referee#2: Page 4060; line 10- This sentence is fairly confusing and reads as if evaporation is a mechanism for releasing bacterial cells a dry aerosol state. "were" should be written as "where". Suggest they rewrite as: "The relative humidity of the chamber was 90 to 95% with respect to ice, thus sprayed droplets evaporated upon entering the chamber. The dried bacterial cell aerosol was then aged for up to 18 hours at the given chamber pressure, temperature and relative humidity, as summarized in Table 1."

Response of the authors: We thank Reviewer 2 for this suggestion, which we integrated in the text (line 113 of the revised manuscript).

Page 4061; line 4 – "...and then saturation with respect to the supercooled liquid droplet phase." should be "...and is saturated with respect to water.".

Response of the authors: This has been corrected.

Referee#2: Page 4061; line 7 – replace "...bacterial cells acted cloud..." with "...bacterial cells acted as cloud..."

Response of the authors: This has been corrected.

Referee#2: Page 4061; line 12 – Please clarify that the chamber was not particle free. Recommend changing "filled with" to "re-pressurized to atmospheric pressure using".

Response of the authors: The chamber was particle free at all times, except for the

bacteria that were sprayed into it. This information is now indicated in Section 2.1 as "After each experiment, the chamber was cleaned by deep depressurization, and refilled with particle free air, so that the chamber was particle free at the beginning of the next experiment.". When it was repressurized after expansion cooling, particle free air was also used.

Referee#2: Page 4061; lines 22- 28 – Recommend including a table to describe different IN activities for these bacterial strains for ease of read.

Response of the authors: As indicated, the information concerning the ice nucleation activity of the 3 bacterial strains used in this study was from previous work (Attard et al., 2012; Joly et al., 2013). Data is already presented in the form of such table for 13b-2 and 32b-74 in Joly et al. (2013). Since the main findings for the 3 strains are summarized here in a few lines (onset temperature of IN activity and frequency of active cells at characteristic temperatures), we think that including such a table is not really appropriate.

Referee#2: Page 4062; line 8 – "as described" where? I think they mean "as described in Section 2.4"?

Response of the authors: This has been changed to "as describe in Section 2.4" as suggested.

Referee#2: Page 4062; lines 17-19 – The wording here is confusing, suggest authors clarify that the control was impingement liquid placed in the impinger without aerosol sampling.

Response of the authors: This was modified into "Unexposed aliquots of the water used as the impingement liquid served as negative controls for ice nucleation assays and cell counts", line 183 of the revised manuscript.

Referee#2: Page 4063; line 1 – Is this assumption correct? Please provide a reference. Is there a size-dependence to the collection efficiency of the impinger (eg. can

the impinger collect a 100 nm particle as efficiently as a 5000 nm particle?)? Please address.

Response of the authors: The assumption that the collection efficiency of BioSampler impinger is 100% was used for relating the concentrations measured in the impingement liquid to the air inside the chamber. Our main constraints in the choice of the sampler here was to use a method largely spread in bioaerosol studies, to avoid altering cell viability and integrity upon sampling, while having good collection efficiency. Impingers were found to be the most appropriate samples in our case. They are widely used for studying airborne bacteria and fungi (e.g. Angenent et al., 2005; Fierer et al., 2008). However, no information was found concerning their actual absolute collection efficiency. Jensen et al. (1992) already used these samplers as the upper reference in a comparative study about the collection efficiency of bioaerosol samplers. It was maximum for particles of 1 μm in diameter, and the collection efficiency was decreased by onlyless than 20% in the case of 0.3 μm particles. It was found that cells can be damaged upon long sampling periods (i.e. > 60min, Terzieva et al., 1996), but they systematically appear as the most appropriate methods for living bioaerosols collection, especially over short sampling times like in our study (10 min) (Griffin et al., 2010; Thorne et al., 1992). The reference Jensen et al., (1992) was included in the manuscript for justifying the assumption of 100% collection efficiency (line 194 of the revised manuscript), and the reference Terzieva et al. (1996) was included for indicating that cell aggregates may have disrupted upon sampling (line 261 of the revised manuscript).

Referee#2: Page 4064; header – should this be INP (rather than IN) assays? Also, please provide a description of how you calculate the error bars presented on Figure 4. Why are some points in Figure 4 without error bars?

Response of the authors: "IN assays" refers here to "Ice nucleation assays", so it is correct here. The error bars were standard deviations from the mean of independent replicate experiments, so these were not possibly calculated in the cases where exper-

iments were not replicated i.e. for example in the presence of ammonium sulfate). The information about error bars was already indicated in Figure 4's legend.

Referee#2: Page 4065; Section 3.1 – Interesting results and the translation to an atmospheric perspective is great and useful. However, I think that if these results are presented this way, there should be a discussion on the caveats of the jump that is made from these lab experiments to the complex natural population of atmospheric bacteria. It is important to translate these results to an atmospheric context, but there is a significant amount of discussion that should be included to address how the translation could be invalid. Although the species evaluated in this study were identified in atmospheric samples, the impact of evaporation (during aerosolization process in these experiments), cloud activation, etc could potentially differ depending on the origin of the bacteria.

Response of the authors: We agree that the experimental set up is probably quite different from what happens in nature. Such discussion about the impacts of other atmospheric factors that could alter survival (i.e. UV light, osmotic shocks and free radicals) was already present in the original manuscript (now in Section 3.4 of the revised version).

I also recommend the authors to reorganize this section. Suggest having subsections for "Time dependent survival rates" and "Impact of cloud processing on cell survival rates" and "atmospheric implications".

Response of the authors: We agree on the fact that Section 3.1 is probably too long and that it mixed different results together. So it was cut into "3.1 Initial total and cultivable airborne cell concentrations" (lines 244-270 of the revised manuscript), "3.2 Survival rate time dependence" (lines 272-308), "3.3 Implications for airborne bacteria dissemination" (lines 310-350) and "3.4 Impact of cloud processing on survival" (lines 352-373). The structure of the next section "ice nucleation activity" remained unchanged, but is now consequently numbered 3.5 (line 375).

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