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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Direct quantification of total and biological ice nuclei in cloud water" *by* M. Joly et al.

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3709, L 26-27, The authors state that "despite low emission rates disconnecting the concentration of INA bacteria existing at the surface of plants from their concentration in the air above (Garcia et al., 2012): : :: : : ". It should be noted that the data of Garcia et al are not proof of low emission rate. The lack of detection by these authors can be readily explained by the relative insensitivity of their technique, which is much less sensitive than classical microbiological methods that were used many years previously by Lindemann and coworkers to detect INA bacteria in aerosols and for measuring flux. The comparative calculations of these methods are presented in detail in :

Morris C.E., Conen F., Huffman J.A., Phillips V., Pöschl U., Sands D.C. 2014. Bio-

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precipitation: Feedbacks linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere. Global Change Biology 20:341-351 (doi:10.1111/gcb.12447).

Morris C.E., Monteil C.L., Berge O. 2013. The life history of Pseudomonas syringae: linking agriculture to Earth system processes. Annu. Rev. Phytopath. 51:85-104.

Garcia et al. (2012) stated that “In contrast to the vegetation, airborne ina gene concentrations at the organic farm were typically below detectable limits, demonstrating a disconnect between local vegetative sources and the air above them.”. However, we do agree on the fact that the method they used (qPCR targeting the ina gene) is much less sensitive than conventional droplet freezing assay, as indicated on Figure 3 of this reference. So, the statement that a disconnection exists between the concentration of IN on potential sources and the actual airborne IN concentrations has been deleted (Line 70).

P 3716, L 26 onward, The authors state: “Lysozyme is indeed responsible for the lysis of peptidoglycans (hydrolysis of the 1,4–linkages between N-acetylmuramic acid and N-acetylglucosamine) and thus specifically targets Gram-positive bacteria. So far, all INA bacteria described in literature including those encountered in clouds were Gram negative species (Cochet and Widehem, 2000; Joly et al., 2013) and they are thus expected to be insensitive to lysozyme. This was verified on 2 of our cloud samples and on laboratory cultures of INA Gamma-Proteobacteria isolated from cloud water (those reported in Joly et al., 2013): lysozyme had no effect on the freezing profiles (not shown).” All walled bacteria, including Gram negative and Gram positive bacteria have peptidoglycan with the linkages described above. Lysozyme is regularly used in molecular biology to lyse cells of Escherichia coli, a Gram-negative bacterium. In our experiments with Pseudomonas syringae, lysozyme markedly reduced INA of strains grown in the laboratory. Furthermore, lysozyme also hydrolyses the 1,4-_-linkages between the Nacetyl-D-glucosamine residues in chitodextrins, a component of many fungal cell walls and can markedly influence the INA of certain fungal spores (Morris et

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al, 2013). However, as the authors noted, the effect of lysozyme is not reliable, but not necessarily for the reasons that they have noted.

Morris C.E., Sands D.C., Glaux C., Samsatly J., Asaad S., Moukahel A.R., Gonçalves F.L.T., Bigg E.K. 2013. Urediospores of rust fungi are ice nucleation active at > -10°C and harbor ice nucleation active bacteria. Atmos. Phys. Chem. 13:4223-4233.

We are completely aware that all walled bacteria have peptidoglycans. However, we did observe on laboratory controls that lysozyme had no effect on the survival and INA of Gram-negative bacteria (Pseudomonas spp.), whereas it affected Gram-positive bacteria's (Arthrobacter sp.) survival when used under similar conditions. In addition, it was reported that lysing Gram-negative bacterial cells with lysozyme, contrarily to Gram-positive species, requires specific pre-treatments such as acetone (Repaske, 1956; Masschalk and Michieis, 2003). As indicated, lysozyme treatment has not reliable effects. So, rather than affirming that lysozyme "specifically targets Gram-positive bacteria", we rephrased the sentence as: "Lysozyme is indeed responsible for the lysis of peptidoglycans by hydrolyzing the 1,4-β-linkages between N-acetylmuramic acid and N-acetylglucosamine, so it is particularly active towards Gram-positive bacteria; its efficiency towards Gram-negative species is much less marked and it requires additional treatments incompatible with droplet freezing assays (Repaske, 1956; Masschalk and Michieis, 2003). So far, all INA bacteria described in literature including those encountered in clouds were Gram-negative species (Cochet and Widehem, 2000; Joly et al., 2013). We verified lysozyme efficiency in altering INA of bacteria on 2 of our cloud samples and on laboratory cultures of INA Gamma-Proteobacteria (Gram-negative) isolated from cloud water (those reported in Joly et al., 2013): lysozyme had no effect on the freezing profiles (not shown). So, this treatment was finally judged not reliable enough here for suppressing specifically bacterial INA and it was not applied further."

Masschalck, B. and Michieis, C.W. (2003). Antimicrobial properties of lysozyme on relation to foodborne vegetative bacteria. Critical Reviews in Microbiology 29:3, 191-214. Repaske, R. (1956). Lysis of Gram-negative bacteria by lysozyme. Biochem.

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Biophys. Acta 22:1, 189-191.

P 3719 L 1-4, The authors state: “If confirmed, such an overrepresentation of high temperature INA cells in cloud water compared to other places in nature would raise the question of the existence of a particular link of ice nucleation active microorganisms with these environments.” For this sentence they are referring to their estimate: “between 0% and about 1.5% of the total bacteria were IN at -10°C.” This remark raises 2 questions. Firstly, do they really mean “overrepresentation” for situations where INA bacteria are 0% of the total population? Secondly, what other environments are they referring to that have been studied in a comparative way ? For the example of P. syringae, when strains that are INA cause disease on plants, this leads to situations where 90-100% of the bacterial population on the diseased plant is composed of INA bacteria. Even as epiphytes, INA P. syringae and Erwinia herbicola (now called Panteoa agglomerans) can constitute well over 1.5% of the total epiphytic population. (Lindow et al, 1978, as cited in the manuscript). Even in the near-surface atmosphere, INA bacteria active at -10°C can constitute over 1% of the total bacteria (Lindemann et al, 1982, as cited in the manuscript). Overall, I think that their proposition of the particular link of INA bacteria with clouds– more so than with other environments - is not founded. Figure 1 illustrates rather well that INA bacteria are found everywhere, and in particular in link with the water cycle. The information in the figure is not presented in such a way that allows one to readily conclude about relative abundance of INA bacteria, but the relevant information for some of these habitats can be found in the publications the authors cited.

We agree that the term “overrepresentation” was not appropriate as this referred to an inferred maximum possible value, rather to an actual value. The corresponding sentence has been removed. In addition, it is right that INA is a rather common phenotype worldwide in nature. So, we do have to acknowledge that since our dataset did not allow to conclude on the actual abundance of INA bacteria in clouds, this sentence could have appeared too speculative. This part of the study was intended to provide

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upper maximal values, but the uncertainty is quite large due to the assumption made that all heat sensitive IN are biological and that all biological IN are bacteria. So, this sentence has been deleted from the manuscript.

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