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Comment

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

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P 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 :

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

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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- β -linkages between the N-acetyl-D-glucosamine residues in chitodextrins, a component of many fungal cell walls and can markedly influence the INA of certain fungal spores (Morris et 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

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and harbor ice nucleation active bacteria. Atmos. Phys. Chem. 13:4223-4233.

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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 *Pantoea 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.

Interactive comment on Atmos. Chem. Phys. Discuss., 14, 3707, 2014.

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