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Interactive comment on "Spores of most common airborne fungi reveal no ice nucleation activity" *by* B. G. Pummer et al.

Anonymous Referee #1

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There is currently a very high interest in the field of atmospheric sciences biological ice nuclei and their impacts on clouds and precipitations. A number of studies reported that bacteria were the most active IN in the atmosphere. To my opinion, such emphasis on biological and microbial IN may have biased the vision of scientists of the atmosphere other than biologists that in reality most of the microorganisms are NOT IN active. This paper reports the ice nucleation activity of a number of fungi relevant in aerobiology. Only 1 species out of 26 investigated here demonstrated significant IN activity, and it is probably the main result of this work. However, the knowledge that is gained from it are rather limited, and one can wonder about the relevance of the fungal strains investigated since they were actually not isolated from atmospheric samples. One of my main concerns about this work is the quantity of spores used in the assays. It was apparently normalized to 20 mg/mL, but there is neither mention about how this

concentration was achieved (by weighting dried spores after collection??), nor to the approximate number of spores it corresponds to. This is crucial since heterogeneous freezing of supercooled water is a matter of probability and it strongly depends on the number of freezing sites available. In the most efficient IN active bacteria, about only 1% of the cells are actually IN. It would have been necessary here to quantify the number of spores or the number of IN sites in the suspension for normalizing the results and be able to compare strains with each others. Using the T50 values might not be relevant since the spore concentrations might have been quite variable from a suspension to another. It could even have not been sufficient to detect any significant IN activity since some droplets may have remained free of any IN spore in the case were concentrations too low were used, i.e. if a weak (which is different from absence) IN activity exists. Hence, I agree with Reviewer 1 that the possibility that IN activity remained not detected due to experimental issues should be discussed in more details. IN addition, more details concerning the determination of IN activity should be given: how many droplets counted? What was the method of counting? Was cooling stopped and temperature steady during counting? What was the reproducibility of the results? Quantification of spore material was attempted by determining total protein content in spore suspensions. This was not correlated with IN activity, and Figure 2 appears quite useless. From the pictures showed in Figure 1, the size of the spores varies quite widely from a species to another, and the protein content itself also varies from a species to another. Furthermore, the spores were harvested from 2 independent plates, in ethanol for IN assays, and in water for protein concentration measurements. The authors seem to assume that those 2 methods lead in similar collection efficiencies of spores from colonies, but this has apparently not been verified? This also has to be discussed, and a few additional experiments for verifying this could be a real plus. Finally, while the problematic of the presence of biological IN in the atmosphere was clearly exposed in the introduction, this is not even mentioned in the conclusion. It would be interesting to indicate what those results imply for ice nucleation in the atmosphere.

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