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Comment on acp-2020-1229

Anonymous Referee #2

Referee comment on "Cultivable halotolerant ice-nucleating bacteria and fungi in coastal precipitation" by Charlotte M. Beall et al., Atmos. Chem. Phys. Discuss., https://doi.org/10.5194/acp-2020-1229-RC1, 2021

General comments:

In this study, the authors collected aerosol and precipitation samples in a coastal environment for INP measurements and cultivation of microorganisms. The microbial isolates from aerosol and precipitation samples were tested for ice nucleation activity. Identification of the isolates was done by DNA sequencing and data base comparison. Phylogenetic analysis, trajectory analysis, and cultivation in marine medium were used to obtain clues for possible marine origin of the isolates. The authors successfully obtained 47 different isolates by cultivation. They found ice nucleation activity in 14 isolates, of which 12 were bacteria and 2 were fungi. Eleven (9 bacteria, 2 fungi) of the 14 IN isolates were derived from precipitation samples and three isolates (bacteria) originated from the aerosol samples. The high fraction of different IN isolates, in particular from the precipitation samples, and number of species with so far unknown IN activity is surprising. Considering the overall limited cultivability of microorganisms under lab conditions, the results of this study indicate that microbial IN activity is far more distributed than previously assumed. The study is suited to the scope of the journal. The method is solid and the results are well presented. The manuscript is well written and well-structured and can be further improved as suggested below.

Specific comments:

Line 25: Better use INP as introduced in line 19 instead of “IN forming particles” as IN is not introduced here and based on IN definition in line 58 it would mean “ice nucleating forming particles”

Line 108: How were the filters pretreated for decontamination before aerosol sampling? Information on blank samples for aerosol sampling and handling should be added.

Line 124: Please add here also the aerosol samples. Moreover, I suggest to add the information given in line 140 about the volume (50 µL) and number of aliquots (30) already here as “microliter aliquots” covers a wide range of possible droplet sizes.

Line 155-157: Please add full information (or a reference) for the performed PCRs (PCR components, concentrations, cycling conditions). Note also, that ribosomal DNA in fungi is 18S and not 16S. Which primers were used for amplification of fungal 18S or did the

bacterial primers only coamplify fungal 18S? This part needs some clarification on how the authors obtained fungal 18S sequences. The authors should also clarify and correct this in other parts of the manuscript., e.g., caption table S1, figure S11.

Line 360: As Cryptococcus and Metschikowia are not bacteria but fungi, please change caption to "Identities of 14 …IN bacteria and fungi". Overall, both fungal species did not receive much attention in the manuscript although the title and abstract raised some expectations. The authors should add some discussion and comparison with the literature for the fungi they advertise in the title.

Line 424: Remove the "sp." after "syringae" as syringae is the species name.

Line 441: please use "spp." and not "sp." if multiple species are meant.

Line 456: "Gammaproteobateria" – typo in bacteria, and missing hyphen (see line 345 Gamma-proteobacteria, be consistent).

Line 465: Lysinibacillus is not gram-negative. Please correct to gram-positive.

Line 490: Can the authors please add some more discussion and more specific suggestions on the "state-of-the art sequencing approaches" they mention here. I wonder how combining INP measurements with state-of the art sequencing should help to identify putative IN microbes that are not recovered by cultivation. The sequencing gives information about composition of the community, which are usually highly diverse, but only a small number of species possesses ice nucleation activity. A diversity analysis, however, does not give information about putative IN abilities of the organisms. Metagenomic (and transcriptomic approaches) are limited by database entries of IN genes, as these genes are not known for the many of the known IN organisms. Also note that some microorganisms (e.g., most known IN fungi) release cell-free IN into the environment. These IN would be covered by the IN measurements but as they do not contain DNA or RNA they would not be covered by the sequencing approaches. Furthermore, without cultivation it seems not feasible to proof the ice nucleation activity of a microorganism, even when (hopefully in future) gene similarities might suggest more candidates.

Table S1: Be consistent - genus and species names should be italics, "sp." should not; contains several typos in e.g., Bacillaceae, Metschnikowiaceae. Paenibacillus is not a family but a genus, thus it should be Paenibacillaceae in the family column. Column blast identity has an extra comma in line Iso39, missing space in line iso3. IN ability column seems not needed, as IN onset temperature gives the "yes" or "no" information.

Table S4: Genus and species names should be italics, Iso5 – missing space, SSA18 – 7tewartia?

Figure S7: Typo in legend: Metschnikowiaceae; what does the line and the Y? at the right side of the legend mean?

Figure 4 and S10: It is confusing that the orange and yellow triangle symbols (sample 9) described in the legend point to a different direction in the plot. Caption for figure S10 needs to be checked "Sample numbers in the legend indication the precipitation"?