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Comment on acp-2020-1229

Anonymous Referee #1

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-RC2, 2021

Terrestrial sources of microbial ice nucleators have been the subject of numerous recent investigations to understand their distribution in and meteorological impact to the atmosphere. Since studies to explore their potential marine sources are surprisingly fewer, this is a research are where more information is needed. This study analyzed aerosol and precipitation samples in attempts to enrich for marine microbes that displayed ice nucleation activity. Despite the biases inherent to this approach for, the authors were nevertheless successful in identifying a number of bacterial and fungal isolates that were tested and shown to be active as ice nucleators at temperatures warmer than -18C, and for one, near -2C. The microbiological and ice nucleating properties of the isolates, coupled with their probable sources in the air masses and precipitation samples analyzed, would be of interest to a broad community of researchers interested in marine and atmospheric science.

One general comment is that the results from fourteen isolates with IN activity are summarized in the abstract by presenting the temperature range of activities from -2.3 to -18C. I believe the truly interesting find in this work is the very warm IN temperature for the Brevibacterium strain, as I am not aware of another report of this phenotype in this phylum. My suggestion is to specifically emphasize this in the abstract as this will be a strain that will elicit interest from a range of microbiologists interested in novel mechanisms of biological ice nucleation.

Below are specific points the authors should consider when revising the manuscript:

Lines 32-33; 93-94; 330-332; 462: The phylogenetic information available cannot be used to definitively determine the environmental source of these isolates. The study observations collectively support that the principal aerosol source was marine, but the type and amount of sequence data obtained do not allow, for example, saying that Psychrobacter sp. 1b2 is marine and 2a is not. The phylogenetic resolution possible from

the V4 region of the 16S/18S rRNA gene, which is about ~1/3 of the gene sequence, is useful for coarse phylogenetic assignments but is not able to resolve evolutionary relationships between closely related taxa. It is safe to conclude that the major source of cultured microbes was from aerosol samples with a marine origin and that many of the isolates are closely related to marine taxa but resolving environmental source from a few hundred nucleotides of small subunit rRNA sequences is not something that can be done with confidence.

Lines 110-111: Just to confirm that the aerosol samples used for culturing in media with ~35ppt salinity were initially placed in deionized water. It is important to note that this process would represent a significant osmotic shock to the cells. Also, assuming that a "hand" was not literally used for this aseptic procedure, so please clarify how the particles were removed from the filters.

Line 144: Just confirming if it was filtered and then autoclaved. Speculating it could be the reverse because a precipitate typically forms when autoclaving full strength seawater. Please also indicate the source of the seawater.

Line 155 and throughout manuscript: "16S V4 ribosomal DNA fragments were…" This is common lab slang that I suggest rewording here and throughout as "The V4 region of the 16S rRNA gene".

Lines 158-159: Suggest editing to something like "The sequences of the amplified 16S rRNA gene fragments were determined by..."

Lines 159 to 161: Please define acronyms on first use and describe the criteria used for OTU designation.

Line 163: Does this mean that the 16S rRNA gene sequences from different isolates were used to create some type of consensus sequence for each OTU? Please explain this process in more detail.

Lines 167-169: The description of this analysis is confusing to me. Distances >0.1 or 10% in the 16S rRNA gene would represent very large phylogenetic distances and not differences that would be confused at being the same "OTU". Please also indicate the length of DNA sequences used in this comparison.

Lines 206-209: It appears that the cells were washed and then tested immediately after nutrient removal. This would provide no opportunity for the microbes to respond to the experimental conditions, so it is difficult to interpret these results as being relevant to the

effect of IN activity on the presence of nutrients.

Line 262: Is each period a separate rainstorm? There is more than one period for some days and with different trajectories in Figure 2, so maybe they are just different sampling periods.

Line 291: Please clarify if the sequence identities in Table S1 are BLAST outputs or based on distance matrices from multiple sequence alignments. Also, a general comment is that a >97% OTU estimate is highly conservative (e.g., Stackebrandt and Ebers 2006, Microbiology Today, 33:152-155).

Line 318-319: The isolated bacterial and fungal taxa cannot easily be compared to microbial communities, which are associations of many many different types of interacting microbes. And if they are Pacific-sourced aerosols, I'm not sure to consider it a "a warmer climate" even though that is the case where they were deposited in SoCal. Can the isolates grow at cold temperatures or is there any other evidence for cold tolerance, if that is in fact what is being implied here? Please revise this sentence for clarity.

Line 334: Is it known if the isolates have optimal growth at salt concentrations in seawater, not just tolerance to the concentrations in the seawater-based media? That would provide physiological support for a marine lifestyle.

Lines 384-385: Does this means than isolates having identical sequences in the portion of the 16S rRNA gene examined were deemed clonal and that one isolate was selected as a representative?

Lines 388-390: Were any of the observations in Figure S11 replicated to confirm that the patterns of IN at these temperatures and isolates were not more affected by the age of cultures or other potential variations in the way the cultures were handled between experiments?

Lines 411-420; 468: Ample time after removal of nutrients may not have been provided in these experiments and the authors should consider limiting this discussion. The one conclusion that can be made is that the activity observed does appear to be associated with the cells and not removed by washing, suggesting the nucleating material is membrane bound or associated with the cell envelope.

Lines 429-432: Please note that the percentage of cells that serve as INPs is temperature dependent, and the "active" fraction values cited from the literature are likely referring to very warm subzero temperatures, whereas for P. syringae populations at temps below

-10C, values approaching 100% could be expected. This leads me to suspect the caveats stated are valid for INPs that activate in the -2C range (i.e., Brevibacterium) but may be overly conservative for the colder temperatures of ice nucleation observed in their experiments.

Lines 436-440: The connection being made with the Failor et al. study is ambiguous. Please clarify if the suggestion is that the taxa in the Failor study were of a marine source, that in the isolates in this study were not marine in origin, or something else entirely.

Line 449: I am not able to find where this is discussed further. This suggests some of the IN activities reported were difficult to repeat, which would be consistent with other similar attempts, Failor et al. being one good example.

Line 484-486: General comment to authors: I am most surprised by the fact that out of a group of less than 50, you found one that is active at warmer than -5C and is a member of a phylum where I am not aware of other known examples of this phenotype. In my view, this single isolate may be the most important contribution of this study and will be of interest for additional work to decipher if the mechanism of ice nucleation differs from that of certain Gammaproteobacteria.

Data availability: Please provide database accession information to access the DNA sequence data from this study.

Figure 3: Please indicate the number of aligned nucleotides and method of phylogenetic analysis used for evolutionary tree construction.