

Dear Editor,

The authors did a great job during their response to the reviewers remarks and significantly improved the manuscript entitled "Microbial and Next Generation Sequencing Approach for Bacteria in Snow and Frost Flowers: Selected Identification, Abundance and Freezing nucleation". Nevertheless:

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• The manuscript is still highly speculative. For instant, the authors discussed the role of *Geobaccter* species in uranium utilization (lines 330-336) but I didn't find any support for presence of *Geobaccter* species presented in the dataset (Table 2; Table 3; Table A.1 and Table A.2). Moreover, throughout the manuscript the authors attempt to infer about bacterial origin and physiological characteristics based on 16S gene similarity, however the percentage of similarity presented here range from 87 to 98%. It is well established that even bacteria with 100% of similarity of the 16S gene, may have completely different metabolic capacities. For instance, *E. coli* strains share 100% similarity however the physiological and metabolic characteristics among them have varied from harmful pathogens to commensal bacteria. Thus I suggest to authors more carefully rewrite the discussion.

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• I have serious concerns about the authors' choice for a DNA extraction method. According the extraction methods presented in section 2.4 (lines 209-213) the authors lose most of DNA from their samples. The 10 minutes of ultra-sonication will disturb the vast majority of bacteria in the sample, releasing genomic DNA into the aqueous phase. Released DNA will NOT precipitate from this phase during a 15 min centrifugation at 18000 g. For DNA to pellet, the solution needed to contain a sufficiently high concentration of salts. Here, the author's will primarily pellet cell wall fragments, denatured proteins and small proportions of undisturbed cells. The DNA extracted using this method represents a tiny fraction of total DNA, only that from undisturbed cells and DNA that had happened to sorb to cell walls.

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• During the results and discussion section the authors did not try to correlate between taxonomy of bacterial isolates and NGS.

• Based on the authors' previous response it is clear that sample triplicates were analyzed.

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*"Each of five snow categories and snow flowers were sampled on different days in multiple replications (at least 3). Respectfully, the results are indeed the representative of three experiments. Hence our sampling and analysis included multiple replicates. This sentence has been added in the revised manuscript"*.

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Nevertheless, it was hard for me to find in the manuscript a clear statement about sample replicates. In case it is triplicates authors must provide values of standard deviation during results presentation, both in text and tables (Table A. 1; Table A.2).

• All published data must be publicly available!!!

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The author MUST deposit the generated sequencing data into a public access archive like NCBI and provide their accession numbers both for NGS and isolates.

WITHOUT THESE ACCESSION NUMBERS WE CAN NOT PUBLISH THE MANUSCRIPT.

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• The pie graph for the Artic blowing snow show that black community represents approx. 75%, however in the related legend its written only 15.28%. Please fix this mistyping mistake (Figure 2 Bacterial community composition in Arctic samples and urban snow at the genus level as detected by Roche 454 GS-FLX Titanium)

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• Section 2.2 line 160 the authors claim:

"To grow the microorganisms, one milliliter of Arctic samples (snow and frost flower) were placed in standard 100 × 15 mm sterile plastic Petri dishes (Fisher Scientific, Montreal, Canada)..."

Figure 3 shows the total CFU/ml above 200. Based on my own experience it is extremely hard to count more than 100 bacteria on the plates, moreover at a higher bacterial density bacteria have competitive interactions. I am assuming that authors performed a serial dilution approach if so please correct accordingly.

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- In Figure 4A it is unclear what the bars represented. The authors write “individual bacterial isolates” however the number of isolates within each snow category doesn’t fit the table 2. For example winpack (WP) in table 2 has only 1 isolate but Figure 4A is represented by 9 bars. Please clarify what bars represents.

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- The taxonomic affiliations of organisms and gene names must be italicized (lines: 28, 36, 178, 266, 302-309, 513-514)

- Line 78 *Pseudomonas viridflava* must be *Pseudomonas viridflava*

- Line 79 *Xanthomonas campestris pathovar translucens* must be *Xanthomonas campestris pathovar translucens*

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- Line 350 *Pseudomonas fluorescens KUAF-68* and *Pseudomonas borealis DL7* must be *Pseudomonas fluorescens KUAF-68* and *Pseudomonas borealis DL7*

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- Please fix the reference list: For example

1. Amann, R. I., Ludwig, W., and Schleifer, K. H.: Phylogenetic identification and in situ detection of individual microbial cells without cultivation, *Microbiological reviews*, 59, 143-169, 1995.

2. Amato, P., Ménager, M., Sancelme, M., Laj, P., Mailhot, G., and Delort, A.-M.: Microbial population in cloud water at the Puy de Dôme: Implications for the chemistry of clouds, *Atmospheric Environment*, 39, 4143-4153, <http://dx.doi.org/10.1016/j.atmosenv.2005.04.002>, 539 2005.

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3. Ariya, P. A., Kos, G., Mortazavi, R., Hudson, E. D., Kanthasamy, V., Eltouny, N., Sun, J., and Wilde, C.: Bio-Organic Materials in the Atmosphere and Snow: Measurement and Characterization, in: *Atmospheric and Aerosol Chemistry*, edited by: McNeill, V. F., and Ariya, P. A., *Topics in Current Chemistry*, Springer Berlin Heidelberg, 145-199, 2014.