**Biogeosciences
Discussions**

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Comment

Interactive comment on "Diversity of bacteria producing pigmented colonies in aerosol, snow and soil samples from remote glacial areas (Antarctica, Alps and Andes)" *by* E. González-Toril et al.

Anonymous Referee #1

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General comments

Overall this paper on the diversity of bacteria producing pigmented colonies living under extreme conditions in glacial areas and high altitudes is an interesting contribution to the expanding research in the field of composition and diversity of microorganisms in air. However, much information concerning the sampling and analyses are missing or poorly described which make it hard to judge how reliable the results really are. Therefore, I would strongly recommend to give the missing information and if not available to perform at least some of the here suggested experiments to make the manuscript and

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the drawn conclusions more powerful.

The most important suggestion by the authors is that the finding of very specific microorganisms in very distant environments is likely caused by air current dispersal. The fact itself that biological particles are transported over long distances by atmospheric circulation is known for a long time, still, the presented research demonstrate a nice strategy to actually give evidence for this fact. However, the sample number analyzed in this project is much too small to be the basis for robust statistics which would be necessary to really give this evidence.

The authors agree that their data cannot rule out the possible model of -everything is everywhere. They are aware that the type of experiments performed cannot rule out contamination. Still, the authors could have done more controls to underline the robustness of their results by trying to cultivate e.g. empty aerosol filters and / or cultivate e.g. snow and soil samples from different not high altitude- samples to show that the results are there different. Especially when trying to deduce important findings from their small sample number, controls are of major importance.

It is known that biological aerosol composition varies according to seasons. The authors do not discuss this issue at all. For the aerosol samples they do not give the details about the sampling time. For snow and soil samples the identification of the season in which the snow fell might be more problematic but maybe the authors could analyze e.g. plant fragments (e.g. pollen) and deduce from them the time of the year when the snow fell.

One major critical point from my perspective is, that it remains unclear for me why so few samples were analyzed and how the sample set was chosen. As it seems more sample were originally sampled but only a subset was cultivated and finally analyzed. As the authors are aware that more robust information would be given by the analysis of more samples, I would strongly suggest to expand the data set if possible.

Specific comments:

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1) As already pointed out in the general comment section, it seems to me a very informative and important aspect to know more about the time and season when the samples were taken/ produced (in case of snow) to be able to judge how well they can be compared to each other at all. Additionally, the questions occurs to the reader why the authors only analyzed five samples when many more seem to have been available. Finally, the way the authors chose their sample remains unclear, in detail:

1a) pg 1610, line 1: As described SEVERAL filters were sampled during 6 years at the coastal Antarctic Station Dumont d Urville. It remains unclear how many samples really were sampled, in which seasons etc. From all these filter-samples just one sample (pg. 1612, line 20), from the 7th July 1999 was chosen for further analysis. Why did the authors choose this special filter? What were the criteria to choose this filter? Why did they only analyze one filter and not more filter from different years, different seasons or the same season and year to look for reproducibility? From my perspective I would strongly suggest to increase here the data set. This would make the manuscript much more powerful.

1b) pg. 1611, line 4: Also concerning the sample number: For both European alpine sites a snow pit was obtained for each. Is it possible for the authors to suggest how old this snow is, from which season it stems?

1c) pg. 1612, line 2: The snow samples were cultured. The authors do not give details how much (weight) snow they cultured. Also here remains the question if it would not have been possible to culture, extract and analyze at least two locations from each snow pit to gain information about the reproducibility or to cultivate and extract both separately but merge the extracts before PCR. This also would represent the microorganism composition in the snow pit better. Additionally, the question occurs how the authors know, how much snow they need to analyze to have a comparable microorganisms density as in the aerosol samples (to be able to compare them in the end).

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1d) pg. 1611, line 9: The same issue holds true for the Bolivian sample where snow blocks were sampled. This also seems to me to be more than one block. Thus, the questions remains here as well, how was the choice done to sample just one block, could more blocks have been analyzed, and is it possible to say anything about the possible time and season when the chosen snow block was produced?

2) pg. 1611, line 22: The authors are aware that contaminations can occur in their methods. However, they do not perform or describe controls, they might have easily done. E.g. for aerosol samples it would be wise to culture or directly extract blank filters as well to observe if microorganisms are directly on the unused filters as it has been shown (in not cited references) that this is possible. Also a necessary negative control would be to try and cultivate pure medium just to ensure that no microorganism ever reached the medium during the laboratory handling.

3) pg. 1614 lines 12-16 and pg. 1615, lines 14-18: The sentences in these two paragraphs are almost identical. The authors should reduce their information to either the material and method section or the results section.

4) pg. 1615, lines 6 and 8: For both, Cyanobacteria and Archaea, the authors did not obtain any positive PCR products. Did the authors test the sensitivity of their primer pairs? Are they able to judge how many Cyanobacteria and Archaea might have been in the sample so that they escaped the polymerase in the PCR? With only 30 cycles and maybe just one try, small numbers of DNA likely escape the amplification process. Did the authors try to reamplify the Cyanobacteria and Archaea from the empty PCR products? This might help to obtain little numbers of products that were not strong enough to be detected with Etbr. Additionally, the question is, whether the authors did positive and negative controls at all. They should give much more technical details here as well. Negative controls are important to show that no contamination during the PCR occurred. Positive controls especially for Cyanobacteria and Archaea are important to demonstrate that at least the PCR reaction worked.

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5) pg. 1615, line 9: The authors state that around 200 sequences were obtained from PCRs with universal bacterial primers. To my understanding an unknown number of PCR products was cloned and in total about 200 clones amplified and sequenced. It would be interesting to give more details here to judge the robustness of the data. How many PCRs with universal primers were done per sample. Just one for each or more? How many clones were picked for each cloned PCR product? Do the 200 sequences represent each of the five samples equally well? Without given details it might be the case that e.g. the samples where only very low diversity was detected were studied based on fewer sequences than other samples.

6) pg. 1615, line 11: The obtained around 200 sequences were compared with the sequences of the public database NCBI. Here also more details would be welcome. How high was identity between the best match and the original sequence? Were they 100% identical? Did the authors add only the first best match to their phylogenetic analysis or several best matches? Did the single sequences give several best matches that were all equally close to the original sequence? How many sequences were finally added to the database used for the phylogenetic analysis and were also here the five analyzed samples represented equally?

7) pg. 1615, line 16: The authors state that filters were used to exclude highly variable positions. Were the filters the reason why they ended with only 26 sequences? What kind of filters did they use? They should also give references why it is appropriate to use such filters in their analysis. The reason I am arguing constantly for giving more details etc is that the final number of sequences obtained is so small. Starting with 200 sequenced and ending with 26 sequences is a dramatic change in the sequence number. With this small sequence finding the data is so much weaker than it would be with more sequences. To judge if there is a real difference between the 2 alpine locations, is with this small sequence number almost not possible.

8) pg. 1615, line 19: From the around 200 sequences that were obtained with the universal primers, finally only 26 sequences remained for the five samples. The authors

do not explain how these 200 sequences were reduced in their number so dramatically. In the phylogenetic tree they used a subset of the 200 sequences. What criteria did they use to create this subset? How many sequences were in this subset? How different were the sequences from the subset? Was each of the five samples represented well in this subset? Why was it necessary at all to work with the subset and not with the complete number of sequences? The authors should give more information here. Otherwise the impression occurs, that they selected the sequences they continued to work with and hold information back which might be interesting for the complete understanding of their work.

9) pg. 1618, line 14: The authors especially concentrate on pigmented bacteria in their title, abstract and of cause their experiments. However, not much discussion is done on pigmented bacteria within the paper thus the reason why the authors restrict themselves to pigmented bacteria remains unclear. Maybe the authors could strengthen this point, in the discussion or change title and focus of their manuscript.

10) pg. 1618, line 19: As this field of research with the genetic analysis of biological particles is growing, the authors should try to catch up with recently published articles dealing also with biological particles in high alpine air.

11) Figures 3-6 and supplementary material: I wondered if it is necessary to present all these trees if they are not discussed in the paper more detailed. Further it would be nice to see the actual bootstrap values to judge how much confidence one can have in the pattern of the tree. Finally more information should be given on the actual calculation of the trees, e.g. how many replications were done, which models were used etc.

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