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Biogeosciences Discussions

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.

E. González-Toril et al.

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Dear Sir: We are sending the revised version of the manuscript “Bacterial diversity of autotrophic enriched cultures from remote, glacial Antarctic, Alpine and Andean aerosol, snow and soil samples” reference BGD 5,S631-S635, 2008, by Amils, Delmas, Petit, Komárek, Elster and myself, for publication in Biogeosciences, in which most of the criticisms and suggestions from the reviewers have been considered. The description of the materials and methods were not clear in the original version, this is probably the reason why many questions and suggestions were raised by the reviewers. In this version we tried to clarify them.

Reviewer #1 - …the sample number is much too small… The intention of the work was not an exhaustive description of the diversity of the systems analyzed, but to identify some of the prokaryotes from remote glacial samples capable to grow in extreme oligotrophic conditions giving pigmented colonies. Obviously more samples would make the analysis more sound statistically, but most of them did not have replicates, and it was not possible to go back to obtain them. We thought that the interest of the results deserved communication. Obviously these studies will we expanded in a near future to probe that restrictive media could be a good methodology to study airborne microbial dispersion, in this case good statistics ir amount of replicates will be required.

- …still the authors could have done more controls….. Controls were made and due to the restrictive conditions none gave a positive result. They have been mentioned in this version of the manuscript.

-…for the aerosol samples they do not give the details about sampling time…Data on how and when samples were retrieved have been introduced in the new version. Extended details are given also in the reference Elster et al., 2007.

- …I would suggest to expand the data…As mentioned this will be impossible for many of them, still be believe that the results are meaningful considering the origin.

Specific questions:

- Question 1). It has been added time of sampling and proposed time of dust transportation. This information was already available in a previous publication (Elster et al., 2007). In any case in has been introduced, as suggested, in this version.

- 1a pg 1610, line1. We agree that a comparative analysis of different aerosol filters will be interesting, but as described this was not the aim of the work. In the new version a detail of how samples were selected is given.

BGD

5, S1412–S1418, 2008

Interactive Comment

- 1b) pg 1611, line 4. Only one sample of snow was available for the work. Details of age of snow and origin of the dust is given in this version.

- 1c) pg 1612, line 2. The requested information has been added in the new version. Cell density in al enrichment cultures was similar; they were incubated until they reach similar OD at 600nm. It is difficult to answer the question concerning comparison between aerosol and snow. To do that quantification data rather than diversity should be used. It could be done with universal in situ hybridization probes, but it was not done because it was not the aim of the work. In any case the question is important and should be considered in the development of further work.

- 1d) pg 1611, line 9. Only one block was available and so analyzed. Details of when was obtained and how samples were prepared are given in the new version.

- Question 2) pg 1611, line 22. Controls were used in the work. They are described in the new version.

- 3) pg. 1614, lines 12-16and pg. 1615, lines 14-18. Redundancy has been eliminated.

- 4) pg. 1615, lines 6 and 8. Cyanobacteria primers work properly because we used routinely in our laboratory. Also they were no detected in the enrichment cultures and in the agar plates. The same is true for Archaea. Positive and negative controls were used in the PCR reactions. Information has been introduced in the new version.

- 5) pg 1615, line 9. Details concerning all the questions about methodology for phylogenetic analysis have been introduced in the new version. Percentage of similarities has been introduced in Table 2.

- 6) pg 1615, line 11. As mentioned, percentage of similarities has been introduced in Table 2. Phylogenetic studies were done with a data base with more than 50000 bacterial 16S rRNA gene sequences by using the ARB program. This data base is updated each time with the best NCBI matches of the obtained sequences. In this way we can garaty that at least 20 nearest matches to every OUT will be present in the data

BGD

5, S1412–S1418, 2008

Interactive Comment

base.

- 7) pg 1615, line 16. When all sequences were aligned we could distinguish different OTUs, thus we could select a representative sequence for every OUT. With these selected sequences we did the complete phylogenetic analysis. Filters are routinely used for phylogenetic analysis to exclude variable positions between sequences in the trees. Every tree is a consensus of more than 16 trees done with 4 different algorithms and 4 different filters. Filters are provided by the ARB software. About 200 sequences were aligned and analyzed but they corresponded to only 25 OTUs, so the number in the phylogenetic analysis was reduced. A note has been added in the new version to clarify this issue. For the evaluation of the oligotrophic diversity of the two alpine samples 50 sequences of each enrichment culture were used. The differences between both samples are real and although we can not explain it, as mentioned in the text, we thought that was important to mention it . - 8) pg. 1615, line 19. This question has been answered in the previous one. The number of sequences is sufficient for a phylogenetic analysis due to the small number of OTUs present in each one.

- 9) pp. 1618, line 14. The title has been changed as suggested.

- 10) pg. 1618, line 19. New and actual references have been introduced in this version.

- 11) Figures 3-6. We think that these figures can help to the reader to evaluate the phylogenetic value of the results mentioned. We agree that not all are needed, but we like to maintain them because there are not too many and they illustrate the similarity detected between sequences of microorganisms from very distant localities. No bootstrap values are introduced in the trees because they are consensus trees.

Reviewer #2

- General comment. As mentioned above, logistic limitations are responsible for the lack of redundancy in the analysis. In any case the work does not pretend to be a definitive study but a preliminary one, suggesting that this type of analysis could be

BGD

5, S1412–S1418, 2008

interesting to study airborne microbial dissemination. The title has been modified, as suggested, and in any case we did not pretend to explain the complex world of microbial dispersal but to contribute with some interesting data concerning oligotrophic microorganisms and the suggestion of its use as a methodology to study airborne microbial dispersion. A short note was not possible due to the information requested by both referees.

- Specific comments:

- …inadequate detail is provided….All the requested information has been introduced in this version.

- ….overall , is the context for sampling…We think that is clear from the text that all results concern recently deposited or actively dispersed microorganisms. Concerning the question of whether an at random sample will be more appropriate for airborne dispersal analysis, there is only one way to answer it, to compare the results, but this was not done because was not the aim of the work.

- … did you balance sampling… There was a balance in the amount of snow used to compare the Alpine and Andes localities. With soil and aerosol to seek for a balance was more complicate, but we think that the amount of generated OTUs from each locality, taking in consideration the extremely restrictive enrichment protocol used, is a good indication that is a fair comparison.

- … what was the function of …. In our experience glass beads facilitates the growth of cyanobacteria and eukaryotic microalgae, which was the original aim of the enrichment cultures. Obviously the lack of growth of these microorganisms make them unnecessary.

- ….other than noting….. We added in the new version the percentage of pigmented bacteria detected in the cultures. These observations were done by microscope analysis (see more information in Elster et al., 2007).

BGD

5, S1412–S1418, 2008

- ….Fig 1 seems…. We think that it is important to show the appearance of the type of bacteria studied in this work.

- …. The statement of objectives…The title has been changed as suggested.

- ….in the first paragraph of the results…. We think that is important to mention that the expected results, the growth of photosynthetic microorganisms, did not happens, and that instead other type of microorganisms, mainly pigmented bacteria, grew in the extreme oligotrophic conditions of the enrichment cultures.

- …due to the characteristics…. We think that the locations where the samples were obtained from and the phenotypic properties of most of the enriched microorganisms in very strict oligotrophic conditions warrants to refer to their idiosyncrasy. This is the reason why the phylogenetic analysis on these samples was performed. The number of sequences was enough to describe the main OTUs present in the samples, this is so probably as a consequence of the low level of diversity due to the restrictive conditions imposed to the enrichment cultures. We tried to identify this type of microorganisms, not to describe the diversity existing in the samples.

- …you suggested….We tried to isolate cyanobacteria and algae from the Artigas sample using an oligotrophic media, instead we obtained only one type of non-photosynthetic pigmented microorganism: only one OTU out of the fifty sequences analyzed. We are claming that our enrichment culture is pure, not the original sample. It could very well be that there are other types of microorganisms in the sample ,but they could not grow in the conditions used in this work.

- …p.1618 top of the page… The referee is right. The numerical data have been removed, we kept a reference to the locations in which the reference samples were isolated.

- …among the 200 total sequences… Cell density was not measured but

BGD

5, S1412–S1418, 2008

Interactive Comment

the enrichment cultures were incubated to reach similar high optical densities. Aliquots of enrichment cultures, no colonies were used for the phylogenetic analysis. This was not clear in the old version but it has been clarified in this one.

- …pigmentation… We can not say that all the enriched microorganisms are pigmented, but we can say that most of them are. We have changed the title of the paper and also corrected the sentences that could induce to mistake.

- p1618, l.25 The referee is right. Even though in situ fluorescence hybridization analysis showed that bacteria were attached to dust particles no quantitative data have been generated. The sentence has been changed in the new version accordingly.

- p 1619. The referee is right. The sentence has been omitted.

- p. 1619, final sentence. Is a statement of interpretation based on the obtained results. We suggest a protocol based on the identification of very specific microorganisms that could grow only in extreme selective conditions, which might help to prevent growth of common contaminates that complicates the interpretation of airborne dissemination studies. Although we can not rule out any other means of dissemination, we consider that airborne dissemination is the best explanation for the Alpes and Andes data.

- Comments on Figures and Tables. - Figure 1. We think that a view of pigmented microorganisms that are studied in this work is pertinent. - Figure 2. The figure shows the phyla in which the microorganisms identified in this work appear. We think that is relevant to the work. - Figure 3-6. The figures are not complicates, they are showing the pylogentic adscription of the microorganisms identified in this work. We think they are important to show that distant localities shear the same type of microorganisms. Table 2 refers to phylogeny but we think is important to see the actual data to illustrate the quality of the work. As mentioned above probably not all are needed, but we prefer to keep them because they are not too many.

Interactive comment on Biogeosciences Discuss., 5, 1607, 2008.

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