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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.

Anonymous Referee #2

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General comments: This paper presents a modest set of data, primarily 16S sequence data, from samples collected from remote glacial areas around the globe. The sampling is inadequate for drawing rigorous conclusions about the differences or similarities in microbial communities among locations. Though the title and introduction emphasize pigmented colonies, the data are not strictly (or even predominantly) for pigmented microbes. The authors draw conclusions about microbial dispersal that do not seem warranted by the results. The contribution of sequence data from remote glacial regions is of value to the scientific community, and will help microbial biologists as we continue

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to develop an understanding of the global patterns of microbial distribution. Overall, however, the results from this work seem more appropriately placed as a poster or presentation at a scientific meeting, or perhaps a short note.

Specific comments: Inadequate detail is provided on the conditions of storage of material, and on material collection. How were filters stored between 1994 and 2000? What time of year were samples collected? How many filters did you examine in this work, corresponding to what time periods? For the snow pit samples, how did you collect the snow—how much snow, from what depths, at what times of year, how did you select the precise location for each sample? The implication (line 8, p. 1611) is that snow sampling focused on recent dust deposits. Is this true, and is it true for all the sites?

Overall, is the context for sampling in all locations to focus specifically on recently deposited or actively dispersing microbes (air sampling, recent dust storms)? If so, this should be explicitly stated. Also, if this is the basis for sampling, this confounds your conclusions at the end that airborne dispersal is critical. If you are specifically sampling locations in extreme environments where there is evidence (e.g. dust deposition) for airborne particle deposition, then it stands to reason that your results will reflect airborne deposition. Wouldn’t a random sample (without regard to visible patterns of dust deposition) be more appropriate for exploring the potential origins/dispersal mechanisms for microbes in remote or extreme locations?

Did you have balanced sampling for 16S sequence analysis among locations? That is, did similar effort go into each location? How did you verify this? Did you sample a similar volume or similar density or some scaling factor among habitats?

What was the function of the glass beads in the culture bottles? (P. 1612, l. 4)

Other than noting that the selection focused on pigmented colonies, can you provide any information at all on the relative frequency of pigmented vs. nonpigmented colonies, or the numbers of colonies of any kind obtained using the two different cul-

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turing approaches from multiple sites?

Figure 1 seems inappropriate in the methods. It does not add much.

The statement of objectives focuses on pigmented micro-organisms (p. 1610, l. 15-20), but only the 5 cultured microbes are documented to be pigmented. The molecular analyses focus broadly on members of the Bacteria, Archaea, and Cyanobacteria. Likewise, the title emphasizes ‘pigmented’. Unless there is something that you have not clarified in the methods, the clone library/16S sequence data are NOT specifically about pigmented microbes. The title, methodologies, and results/discussion need to accurately reflect this fact!

In the first paragraph of the results, you note that you have used a protocol for enrichment of photoautotrophic microbes. Yet it is also clear from your results that photoautotrophic microbes are not the ONLY microbes that can grow in this low-nutrient medium (and in fact your DNA results suggest that you didn’t have any phototrophic bacteria in your samples), and your molecular approaches targeted bacteria, archaea, and cyanobacteria. Thus, the following sentence seems doesn’t really make sense for this paper:

“Due to the characteristics of the cultures and the idiosyncrasy of the samples it was considered of interest to identify the microorganisms present in the different cultures, to evaluate the diversity corresponding to this very specific type of microorganisms, and eventually to compare the results obtained in the geographically dispersed sampling sites”

What characteristics of these cultures are significant? What is meant by ‘idiosyncrasy’ of the samples? What very specific type of microorganism do you refer to? And the sampling intensity (total of 200 sequences over all sampling sites) is inadequate for a rigorous comparison of communities among sites. Overall, the sentence seems like a misleading description of the results presented here.

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You suggest that the Antarctic enrichment culture was a pure culture because you isolated only one sequence from this sample. However, you do not note in your methods the volume of the enrichment culture from which DNA was extracted. Presumably you didn’t extract DNA from the entire enrichment culture. What is the fraction of the medium/culture that you extracted, and what is the likelihood or possibility that there were other organisms in the non-sampled culture?

p. 1618, top of page, the following sentence doesn’t make sense: “The distribution of detected bacteria was related with cold environments (21.4%), marine environments, ... “ etc. Do you mean specifically that, of the 200 sequences detected, 21.4% were found in cold environments, 35.7% were found in marine environments, and 35.7% were found in soils/subsurfaces? What is the relevance or significance of these percentages, since the collection of 200 is an arbitrary assemblage?

Among the 200 total sequences, do you have any information on how well you have sampled each community? Specifically, do you have data on microbial densities in the culture bottles or on the plates? You selected 5 pigmented colonies for phylogenetic analysis. Out of how many total colonies? How many total pigmented colonies? How many cells/ml in the glass bottle cultures? What proportion of the total number of microbes did you sample? How did this vary among locations? These are critical to understanding the effectiveness of the sampling for describing and comparing communities. These sorts of information need to be included to the extent possible in this paper to help the reader.

Pigmentation...at best you have a weak statement to make (p. 1618, l. 14-18). This paper is not really about pigmentation or pigmented microbes in any exclusive way.

P. 1618, l. 25: You have no data at all on attachment to dust particles! On what do you base this statement??

P. 1619: Why do the location of the sampling sites and the results rule out contamina-

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tion? What about contamination in the laboratory? Why is the variation between the 2 samples from the Alps a useful internal control for lack of contamination? What if one of the samples was highly contaminated and the other one was not?

p. 1619, final sentence. “We strongly believe that the common microbial patterns of peculiar microorganisms observed at distant locations reflects truly airborne microbial dissemination, so we will like to propose this protocol for further testing this model of microbial dispersion”.

Is this a statement of belief or is this a statement of interpretation based on your results? I think you need to present a more compelling case that your data support the hypothesis of airborne microbial dissemination as an alternative to other possibilities. Also, what do you mean by 'this protocol’? How specifically does the approach that you have taken in this work test 'this model of microbial dispersion’? By 'this model’ do you mean airborne dispersion? If you find a microbe in soil or in snow, how does this reflect its mode of movement? What about movement IN snow or IN water. Is this synonymous with airborne dissemination? I am not convinced that your approach rules out one form or another of dispersal.

Comments on Figures and Tables:

Figure 1: does not add significantly. What are these isolates? Where are they from? What is their significance relative to this work?

Figure 2: Are these pigmented phyla ever having been identified anywhere? How does this relate specifically to this study and your results?

Figures 3-6: Very complicated figures with little information about your specific samples. Each of these figures have at most 3 of your isolates represented within a large collection of isolates from other studies. Table 2 provides the critical information more effectively.

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