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Biogeosciences Discussions

Interactive comment on "Transport and characterization of ambient biological aerosol near Laurel, MD" *by* J. L. Santarpia et al.

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GENERAL REMARKS In this study the authors aim to develop "a method for understanding and comparing the molecular and culturable prokaryotic diversity in the ambient aerosol background". To accomplish this they proceeded by "comparing the culturable fraction and surveying the total 16s rRNA of each sample; [this] provides a comprehensive look at the bacterial population studied and allows comparison with the broad range of previous studies."

With the growing interest in the microbiology of the atmosphere - either for the purpose of understanding dispersion of human or plant pathogens, or to investigate the impact of micro-organisms on chemical and physical processes in the atmosphere – this goal is pertinent. Both approaches to describing and quantifying the diversity of the aerial

microflora have their advantages and disadvantages. Techniques based on culture media for characterizing the diversity of the aerial microflora were the only techniques used before the advent of molecular characterization. But they continue to offer certain advantages and they will certainly not be abandoned. These two families of techniques will continue to co-exist and hence we need some ways to "translate" results to allow each of these approaches to fully nourish the body of information on dynamics of micro-organisms in the atmosphere.

To compare diversity between approaches, I would have expected the authors to at first establish criteria for comparison that would take into account the sources of variability and the detection limits of the two families of techniques or that would have in some way tried to quantify results so as to be able to make critical comparisons. It is not clear what hypotheses were tested here. The authors seem to argue that, overall, the two different approaches give similar information about the abundances of Gram- and Gram+ bacteria (if we make certain assumptions), but this is not clear. It is also not clear how the phenotypic data, other than Gram reaction, is compared to the results from molecular analyses. I would also expect that the results to be compared would both issue from random samples. But the strains collected were pre-screened based on morphological and phenotypic bases rather than being collected at random from colonies growing on plates. This makes it confusing to understand what criteria were used to compare the results of the two approaches and how this could be useful for analyzing results of other studies.

The discussion focuses quite a bit on the potential origin of the bacteria in the samples, but it does not go beyond any current knowledge in this regard. They do not state in the introduction that understanding transport is an objective. More specific remarks about these comments are made below:

SPECIFIC REMARKS

LINE 135-136 : "The MT is set according to:

BGD

7, C3338–C3344, 2010

where N was the noise value generated for each Phylochip during processing (Brodie, et al., 2007)."

Is something missing here (after the colon)? Or do I have a problem with the pdf reader?

LINES 162-165 (Results): "Phylogenetic data from all four aerosol collection periods on the 4th and 5th (Figure 2, Table 1) indicate that the biological diversity is increasing over the course of the 4 collection periods."

How can the authors differentiate between i) increases in the diversity (in terms of number of taxa) and ii) an increase in the overall concentration of bacteria in the air that would allow detection of taxa that were present at concentrations below the level of sensitivity in the earlier samples? It would have been useful if the authors presented values for the total quantity of DNA collected in each sample. This would give some information about the total biological load of each air sample.

Furthermore, the table and figure are not easy to interpret with regard to conclusions about comparative diversity among the samples. For figure 2 in particular, the pertinent data about the OTUs present in each sample are presented in the tiny bars along the perimeter of the phylogenetic tree and are very difficult to compare. The colors of the taxonomic groups are very visible, yet the identity per se of these groups is not the real message of figure 2 (that information is available in table 1), and therefore it is distracting. I would suggest presenting the data differently. The authors could use the same type of circular phylogenetic tree, but the central colors could represent the % of each taxa for each sample in the total of the OUT's detected collectively. In Figure 1. I have illustrated this in a linear form (with hypothetical data). This would allow an easier visual comparison of the 4 samples.

LINES 181-231 (Results): In the presentation of results concerning taxonomic variability of the strains collected from culture of the samples on media, I was surprised that the authors did not make any direct comparisons of the Phylochip data. I would have

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7, C3338–C3344, 2010

expected 2 approaches. Firstly, it is very surprising that the authors did not determine the sequences of the 16S rRNA genes of the 28 bacterial strains collected. This would have allowed them to assess how these strains would have been classified according to the Phylochip, thereby allowing for a stronger comparative analysis. Furthermore, it is unfortunate that the authors made a preliminary screening of the strains based on physiological and morphological criteria. Their results would have been more powerful if they had sampled strains randomly from plates, eliminated non-bacterial isolates, and determined the 16S rRNA sequences without pre-screening according to phenotypes. Sequencing is relatively cheap and very rapid compared to the classical microbiological analyses. Secondly, in the conclusion the authors make comparisons of the ratios of Gram- to Gram+. However, the fraction of OTUs in the phylogenetic tree that represent Gram+ or Gram – groups should be specified given that Biogeosciences is an interdisciplinary journal and that only a small proportion of readers will know this information without having to consult a reference document. Hence, the reader has no way to judge the validity of the conclusion. They collected strains to be able to compare the results of the 2 types of approaches. Such comparisons have not often been made for the same samples and it allows us how to compare studies of air-borne microflora that use different detection techniques. Hence, the authors should lead us through their observations with more detail.

LINES 241-242: “If the contribution from Proteobacteria is removed from the analysis, since its members are generally considered to be functional anaerobes . . .”

In the Proteobacteria one finds the Xanthomonadales and the Pseudomonadales, for example. There are many members of these families that are not functional anaerobes and are in fact strict aerobes. I do not understand why the authors state this. Hence the logic of their arguments concerning comparisons between data from culture media and from the Phylochip is not clear.

In LINES 251-255 the authors speculate about the possible origins of bacteria in the air based on taxon identity. From this they suggest that the presence of Cyanobacteria

BGD

7, C3338–C3344, 2010

and Planctomycetes indicates an oceanic source of micro-organisms in their samples. Perhaps this is possible, but they forget that these two groups of organisms are also abundant in soils. They do not need this type of speculation to justify the use of HYSPLIT to investigate the origins of the air masses. Nevertheless, to uncover the real origins of the bacteria in the atmosphere will require targeting genes that give information more refined than that contained in the 16S rRNA gene and that can delineate biogeographic patterns and even clonality. Furthermore, due to the massive numbers of bacteria in soil and water and the physical processes known to lead to their transport into the atmosphere, it is already well accepted that these are two important sources of bacteria in the atmosphere. It is not clear how their results re-inforce this understanding.

LINE 259: The authors should explain what they mean by "Initially, the broad diversity of the organisms found in the aerosol samples was alarming." Is it alarming because they were not confident in technical aspects of their work? Or was it alarming because they expected the air to be carrying little microbial diversity? And if so – for either of these points – why did they have this expectation?

LINES 262-264: "The back-trajectory analysis provides a potential explanation of the source of the ribosomal signatures of these phyla. One exciting implication of these findings is that all air samples will carry a biological record of their history."

Is it surprising that air masses in Maryland come from the ocean? The authors should try to situate this information in terms of regular patterns. Furthermore, air masses have chemical records of their history that are usually more telling than the biological records. These chemical records include salts, pollutants, soils of various chemical composition, etc. that are very good and probably more specific indicators of the origin of the air masses. In this light the authors should indicate how the biological indicators could be more informative or offer complementary information.

LINES 266-267: "This study indicates that even under normal weather conditions, ge-

BGD

7, C3338–C3344, 2010

netic evidence of the microbial communities within the path of the sampled air mass may exist."

This statement is not supported by the genetic evidence of their data. Their genetic evidence gives taxonomic information but does not show any link with bacteria in the suspected sources. 16S rRNA sequences can indicate species identity but not the subspecies or clone family identity needed for tracing. Another way to address the question of sources would have been to show a gradient of types along a potential trajectory, but this study did not deploy the experimental set up to be able to show this.

LINES 268-270 "Since the urban environment is likely to have a number of unique microbial communities, this information may be used to help identify the source location of pollutants and provide additional evidence to support attribution of pollutants or hazardous emissions to specific locations."

The authors should explain this a bit more. Why is it likely that urban environments could have unique microbial communities? What types of micro-organisms would constitute these communities? Are there any references for this idea?

Interactive comment on Biogeosciences Discuss., 7, 6725, 2010.

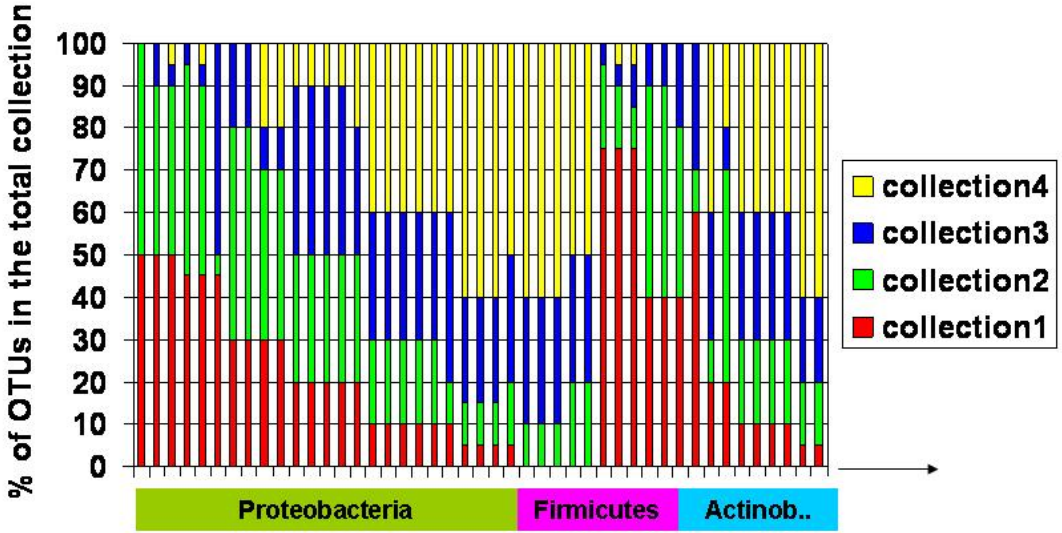

Fig. 1. Figure 1: Suggested approach to presenting data in Fig. 2.

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7, C3338–C3344, 2010