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Biogeosciences Discussions

Interactive comment on "Microbiology and atmospheric processes: biological, physical and chemical characterization of aerosol particles" *by* D. G. Georgakopoulos et al.

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The manuscript by Georgakopoulos and co-workers represents the fruit of one of the working groups that emerged from the ESF workshop on " Microbiological Meteorology " in March 2006. The objective set for this group was to construct a thorough and critical review of the panoply of approaches to characterizing biological aerosols, a review that would be particularly useful for new-comers in this field of research. Overall, the manuscript is well constructed, informative, easy to read, and provides critical appraisals for most of the techniques presented. Furthermore, it is the only compilation of such information of which I am aware. My specific comments are listed below.

1) In the introduction the authors should state that this paper originates from the ESF workshop and that it is part of the series with the other papers with same title prefix. They could follow the example of the remarks in the paper by Deguillaume et al in Biogeosciences Discuss.

2) I was surprised that virus particles were not covered in this manuscript. They have been found in clouds (Castello, J.D., D.K. Lakshman, S.M. Tavantzis, S.O. Rogers, G.D. Bachand, R. Jagels, J. Carlisle, and Y. Liu. 1995. Detection of infectious tomato mosaic tobamovirus in fog and clouds. Phytopathology 85:1409-1412) and are also, in general, known to be disseminated via the atmosphere.

3) References are lacking in some sections. Those sections for which it would be important to add citations of references are:

- In the introduction concerning the size distribution of particles relative to their labels ("nuclei mode", "accumulation mode", "coarse mode"). -Paragraphs 2-5 of the introduction are also lacking citations of references. -Pg 1478, paragraph beginning on line 15, references are needed for statements about inhibition of PCR by salts. -Pg 1487, lines 10-12, statement about the number of copies of the 16S gene per cell.

4) In terms of organization of the paper, it would be more logical to begin with techniques that treat micro-organisms as particles " i.e., flow cytometry and microscopy " before the techniques that examine their biological and/or chemical properties.

5) In the section on cell culture (p. 1472, line 22): biochemical tests of bacteria are less and less used as the sole means of identification. The authors need to add that identification is based on biochemical tests as well as on genetic profiles. In this same paragraph, the authors state that an advantage of culture methods is that they (the Petri dishes) are compatible with many types of air samplers. In addition to this, they should note that these methods are also quantitative. Yes, they underestimate (because of lack of culturability of all cells), but if errors are constant they permit tests of hypotheses that require quantification. And, they also permit recovery of strains for

BGD

5, S540–S544, 2008

further characterization.

6) P 1473, line 9: "a variety of media and incubation conditions"IS necessary.

7) P 1473, line 24: ".. pass through the same hole and land8221; AS AGGREGATES on the agar".

8) P 1473, line 28: The proper term is CULTURABLE, and not "cultivable".

9) Section on microscopy, On p 1474 line 5 the authors present the possibility of sampling directly onto glass slides. However, the presentation seems to suggest that this is the only solid substrate on which samples can be sampled for microscopic observation. However, filters and glass rods (Rotorods) can also be used. Further on they remark that direct species identification is not possible. However, for many species of fungi it is possible to identify them directly. An example of this is the paper by Elbert and colleagues (2007: Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions. Atmos. Chem. Phys., 7, 4569-4588).

On line 14 they indicate that 10e4 bacteria must be present to detect them via microscopy. What are the units on this value: 10e4 cells per what volume or what surface ?

10) Immunological detection: p 1475. In this section the authors make statements based on unpublished data that would discourage anyone from attempting serological detection of the INA protein. I think that this type of opinion should be based on data that readers could evaluate. Without sufficient data, it would be best to moderate the statement. Furthermore, on the next page of the same section the authors suggest that an antibody for the ice nucleation protein would be useful if coupled with flow cytometry. The authors should resolve the contradiction of their opinions in this section.

11) P 1477 line 14: ".. for at least 20 min, seems to" BE the minimum..

12) DNA/RNA isolation, p 1479 lines 19-25: Here the authors cite a technique for isola-

BGD

5, S540–S544, 2008

tion of the DNA associated with live cells via the use of EMA. However, this technique has proven to be efficient only for a limited number of species. A more robust technique that has wide application is based on propidium monoazide (PMA) (A. Nocker et al. 2006 Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J. Microbiol. Methods. 67: 310-320).

13) In section 3.3.2 and onward, the use of the terms 16S rDNA and 16S rRNA is not consistent and is confusing. These terms are often confused among biologists. Hence, it would be useful to add a footnote (or text box) to explain the difference between 16S rDNA and 16S rRNA and in what context each term is used. This is important for readers who are not specialists in molecular biology but who might be using these tools. An example of the inconsistency that I see here is the use of the term "16S rDNA gene" (p. 1481 line 1, for example). Normally the name of the gene indicates the product of interest (in this case: rRNA) and it should be in italics. The authors write "inaW" (italics) for the name of a gene; so why shouldn't the gene for ribosomal RNA be called "16S rRNA" (italics) gene? Likewise, primers and probes (p 1480, line 14) are for DNA and not for RNA. If they include an explanatory footnote, it could start something like: "To manufacture proteins, ribosomes interact directly with RNA (and not DNA). The nature of this RNA, and hence the gene (DNA), is highly conserved because of its important biological role. However, classical PCR amplifies DNA. In phylogenetic studies we target the DNA that codes for the RNA that interacts directly with the 16S or the 18S component of the ribosomes."

14) P 1483, discussion about restriction enzymes and RFLP: Some of the vocabulary here seems like jargon or short-cut "lab talk". For example: is "forward primer" jargon? Is this precision even necessary here to understand the technique? Also: the phrase "because most species possess a cut site near the primer" might be more understandable if it read: " .. near where the primer binds". The sentence "the position of the cut site varies among the different bacterial groups" is confusing. This might lead the

BGD

5, S540–S544, 2008

reader to think that the enzyme does not always cut at the same site. It would be more understandable as: "the position, in the total genome, of the site that is cut varies."

15) On pp 1491 to 1493 the term "biogenic" is used. This should be replaced by "biological"

16) In the Conclusion section the authors use the word "climatic", which should be changed to "climate": "climate changes", and "role in climate".

17) Among the methods covered, they should include the use of lysozyme and of boiling to identify the ice nuclei of biological origin as recently described by Christer et al (2008. Ubiquity of biological ice nucleators in snowfall. Science 319:1214).

Interactive comment on Biogeosciences Discuss., 5, 1469, 2008.