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Biogeosciences Discussions

Interactive comment on "Coupling of fog and marine microbial content in the near-shore coastal environment" *by* M. E. Dueker et al.

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General comments:

We would like to thank Reviewer 3 for constructive comments on our manuscript. We are pleased that the reviewer agrees that our results support the hypotheses outlined in the paper and that the results are carefully discussed. We agree with Reviewer 3 that media-based approaches do not allow all groups of microbes to be cultivated, therefore introducing a bias in the detectable microbes. However, culture-based methods have important advantages, as compared to cultivation-independent approaches, and are a legitimate way to evaluate relative changes in a subset of bacteria capable of growth on provided media. A culture-dependent approach was the only means to assess viable (metabolic machinery intact) bacterial fallout near-shore, which was the focus of the

study. Other culture-independent methods, including pumping onto filters and liquid impingement, do not discriminate between cells that are damaged and incapable of metabolism and cells that retain viability. Knowing that the cells deposited are viable is a very useful piece of information when compared to culture-independent studies, especially in terms of public health and other issues related to pathogen ecology (including human, animal, plant pathogens), but also for understanding the potential for biogeochemical transformations in the air and upon deposition. Therefore, we feel that the benefits of the culture-dependent approach outweighed the inherent biases introduced and that the methodology was well matched to the objectives of our study.

RC = reviewer comment; AC = author comment

Specific comments:

RC1: "The first concern is that the sequencing effort largely differs among 3 types of samples (151 fog, 13 clear, 37 ocean). Small libraries of the 'clear' and the 'ocean' may miss some OTUs, which can change some results (e.g. no of shared OTUs, similarity indices, Venn diagram) and possibly conclusions."

AC1: The size of sequence libraries is always a concern – and there are statistical analyses specifically designed to address these concerns and sampling biases. We used Chao's Corrected Jaccard and Sorenson indices to account for the small size of the clear library in statistical analyses (Chao, Chazdon et al. 2005). Also, small samples can be expected to over-represent numerically-abundant organisms, which did not appear to be the case when fog and clear libraries were compared. These libraries were dominated by different genera (see Tables 2 and 3). Given the limited amount of sequence information available for microbial aerosols in the near-shore environment, especially from cultivated microbial aerosols, the data present in this paper provides a substantial contribution to the field.

RC2: "The second concern is the potential bias caused by the use of specific media for measurement. If the media are more suitable for culturing marine bacteria than

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terrestrial ones, all libraries can be dominated by marine isolates and thus the source of them looks an adjacent ocean surfaces. What is NaCl concentration of the LB used in this study?"

AC2: The Luria Bertani agar (LB) media we used was the Miller formulation, and contained 10 g/L NaCl, compared to 20 g/L NaCl present in Marine agar. We did not expect this media to unduly favor marine organisms over terrestrial because LB is commonly used in prior published culture-based aerosol studies in terrestrial and coastal environments (for example, see Lighthart and Shaffer 1995; Shaffer and Lighthart 1997; Tong and Lighthart 1997). Also, similar work within our laboratory at other sites (sampling at both coastal and inland areas) has demonstrated that a wide variety of both terrestrial and marine bacteria are capable of growing when deposited on LB plates. It is interesting to note that while Reviewer 1 is concerned that LB may not adequately grow marine bacteria, Reviewer 3 is concerned that LB may be favoring marine bacteria. While no media selection is entirely without bias, based on prior publications and data from our own laboratory, we were confident that LB allowed the growth of a combination of marine and non-marine bacteria, making it an appropriate media to address the objectives of our research. Furthermore, the main result of this study derives from the relative difference in cells cultivated under different environmental conditions using the same media.

RC3: "The third concern is about colony PCR. I was wondering how much the success of colony PCR of the 3 libraries. Since terrestrial bacteria are harder to lyse than marine ones, failure of PCR amplification can be a potential bias in describing community composition."

AC3: As with any PCR-based approach for analysis of microbial community composition, there can be biases associated with lysis and the efficiency of amplification. While not all colonies on the plates in this study could be successfully amplified using the lysis and PCR conditions described in the manuscript, we have no data to indicate that this would bias community composition to favor marine bacteria. As mentioned above,

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we have sampled in other terrestrial and coastal sites and successfully amplified marine and terrestrial microbes including diverse Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria in our laboratory using the same methodologies. At some inland sites our libraries are completely dominated by terrestrial bacteria, confirming that the methodologies are capable of amplifying both marine and terrestrial microbes.

RC4: “P9615 line 7 What kind of polymerase is used? Information of PCR enzyme is useful for readers.”

AC4: TopTaq DNA Polymerase (Qiagen, Valencia, CA) was used in this study. This information will be added to the methods section in the revised manuscript.

RC5: “P9615 line 24 No other categories such as human and animal sources?”

AC5: For the purposes of the study we restricted our categories to marine, terrestrial and aerosol sources. Land animal and human sources were included within the terrestrial category for this study, and ocean biota sources were included within the marine category. We will clarify this in the methods section of the revised manuscript.

Technical comments:

RC6: “P9615 line 12 Drummond et al 2010 is missed in the reference section.”

AC6: We will make this correction in the revised manuscript.

RC7: “P9616 line 14 Colwell 2009 is missed in the reference section.”

AC7: We will make this correction in the revised manuscript.

Works Cited:

Chao, A., R. L. Chazdon, et al. (2005). "A new statistical approach for assessing similarity of species composition with incidence and abundance data." Ecology Letters 8(2): 148-159.

Lighthart, B. and B. T. Shaffer (1995). "Viable bacterial aerosol particle size distri-

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butions in the midsummer atmosphere at an isolated location in the high desert chaparral." Aerobiologia 11: 19-25.

Shaffer, B. T. and B. Lighthart (1997). "Survey of culturable airborne bacteria at four diverse locations in Oregon: Urban, rural, forest, and coastal." Microbial Ecology 34(3): 167-177.

Tong, Y. Y. and B. Lighthart (1997). "Solar radiation is shown to select for pigmented bacteria in the ambient outdoor atmosphere." Photochemistry and Photobiology 65(1): 103-106.

Interactive comment on Biogeosciences Discuss., 8, 9609, 2011.