

Interactive comment on “Detection and phylogenetic analysis of coastal bioaerosols using culture dependent and independent techniques” by R. Urbano et al.

R. Urbano et al.

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General Response:

The goal of our project was to get an overview of the types of microbes found in the air at our sampling site and an indication of their possible source of origin (Terrestrial vs Oceanic). From the total of 55 sequences obtained (bacterial + eukaryotic) through four independent air sampling events, we saw a general clustering of prokaryote and eukaryotic sequences. Among the clusters, we detected sequences from several strains that were repeats or belonged to highly related strains. This is indicative that a sufficient number of sequences was collected to provide a bona fide representation of the

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bioaerosols present at our coastal site during our sampling events. Since we did not get a high number of sequences belonging to phyla outside Basidiomycota, Ascomycota, Firmicutes and Proteobacteria (one out of 16 in the 18S rRNA gene tree and 4 out of 39 in the 16S rRNA gene tree), we determined we had enough data to provide conclusive evidence that the mentioned phylum were highly abundant bioaerosols at our sampling site. Had we collected a lower number of repeated sequences or sequences belonging to less related genera, we would have sequenced additional clones. However, this was not the case. Most of our detected strains are ubiquitously found in soils and terrestrial sources; within these, several have been previously associated with marine environments as indicated with asterisks in the phylogenetic trees (citations provided 9. 4-13; additional citations will be provided in the revised manuscript). We cautiously suggest that the marine association of strains could be an indication that important aerosol generating events take place in coastal regions (e.g. beaches).

Regarding concerns with lengthiness, style and content, the manuscript will be reworked to provide a more concise report of our study.

Response to Specific comments:

1. 1, 8-9. You did not investigate the exchange of airborne microorganisms at the air-sea interface. Please, remove.

Response: Item removed, sampling will be referred to as “coastal” throughout the manuscript.

2. 1, 11. Given the limited number of clones and isolates, you are not “determining the microbial diversity”. Rephrase to e.g. “get insights into microbial community composition”. Moreover, the number of clones and isolates obtained should be evident from the abstract.

Response: Sentence will be rephrased and number of clones and isolates added to the abstract.

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3. 2, 17. or instead of and

Response: Sentence will be corrected.

4. 2, 25. To help readers not familiar with this subject, please explain what 0.7-0.11% means.

Response: In the atmosphere, supersaturation denotes a water vapor content greater than 100% relative humidity. A short explanation will be provided in revised manuscript.

5. 3, 29. influenced

Response: Item was corrected.

6. 4, 19. Please, insert the answer to that question.

Response: The sampling site is approximately 12 meters above the ocean.

7. 4, 22. What were the filter diameters?

Response: Filter diameters were ~5cm.

8. 4, 25. In centimeters, please.

Response: dimensions will be changed to 2.4 meters in height and 2.5 centimeters

9. 5, 3. It is unclear what is meant by control filters. What was exactly done? Were they just blank filters run in parallel? Did you try to PCR amplify from these control extracts? It appears so from 8, 17. This needs to be carefully described.

Response: Control filters were treated in a similar fashion to sampling filters, except air was not sampled. The control filters were basically blanks where we attempted DNA extraction and PCR amplification to detect any positive artifacts associated with our methods. Our DNA extraction protocol yielded DNA below our detection levels and no PCR amplification signal was detected.

10. 5, 10. Delete "all"

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Response: "all" will be deleted in revised manuscript.

11. 5, 11. pH?

Response: pH 8.0 will be added to revised manuscript.

12. 5,21. 18s rRNA gene amplification. This needs to be corrected throughout the manuscript. You are not working with 18S/16S rRNA or 18S/16S, but with 18S/16S rRNA genes.

Response: This will be corrected in the revised manuscript.

13. 5, 24 + 6. It is important for the reader to know the fragment sizes you're working with. Please insert position in association with the primer specifications.

Response: The fragment size for both 18S and 16S rRNA genes is given in the Phylogenetic analysis section.

14. 7, 5. Primers, not probes.

Response: Probes will be changed to primers in the revised manuscript.

15. 7, 9-10. What is meant by all data? Response: By "all" data, the authors are referring to all the sequences collected during this study and their respective top sequence matches collected from GenBank. This sentence will be revised to make less confusing.

16. 7, 13-14. This is self evident. I suggest deleting this sentence

Response: Sentence will be deleted.

17. 7. The obtained sequences need to be submitted to GenBank and accession numbers given here.

Response: Sequences will be submitted to GenBank and accession numbers added to the revised manuscript.

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18. The Results section should be consistently in past tense. The whole section needs a complete make-over when comes to language and content. It looks very preliminary and I suspect that the corresponding author did not put effort into this section. That is indeed needed and required to make the text readable, non-redundant, and only contain appropriate information.

Response: Results section will be re-worked to address your comment.

19. 7, 22. Delete methods

Response: "methods" will be deleted.

20. 7, 23. These are isolates and not clones. Revise this throughout the ms.

Response: Item will be Revised.

21. 8,16-17. Exactly how were these tests performed?

Response: We ran a PCR reaction with all reagents minus DNA (negative control) in parallel with the DNA that was extracted from filters; this resulted in no amplification; the authors will add this to the revised manuscript.

22. 9, 13. R.?

Response: Please clarify comment (this refers to *Ralstonia pickettii*)

23. 9, 14. Unclear. Please clarify.

Response: Sentence rephrased to "Air mass back trajectories were analyzed for each of the sampling dates."

24. 9,22-26. Revise sentence. It appears as if you expect fungi sequences by Scripps Pier because they have been found in the tropical rainforest, which doesn't make sense.

Response: sentence will be clarified in the revised manuscript.

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25. 9, 22. phylum

Response: Sentence was revised to "Only one non-fungal sequence was detected; the sequence matched *Pycnococcus* sp. of the Chlorophyta phylum (Viridiplantae kingdom)."

26. 10, 7-9. In principle it is extremely limited what you can say about the composition of bacteria/fungi in the aerosols based on the total of about 40 sequences obtained from isolates and clones. It needs to be highlighted in the text that the conclusions of this work is based on a very small dataset – and the authors need to be very careful with the conclusions drawn. 28. Since sequences representative of common marine bacterioplankton species were not found, the authors suggest that fungi and bacteria in the aerosols originate from sandy beaches. The logic question is then, which bacteria/fungi are known from sandy beaches? I'm sure there are many references to choose from. The authors should make this comparison. Without it the suggestion is just unsubstantiated speculation that should be removed.

Response: The amount of sequences clones and isolates will be highlighted in the revised manuscript. However, repeated sequence and highly related strains in our dataset are indicative that in our sampling conditions certain bacterial and fungi are found in high abundance. Furthermore, our data clearly shows a number of sequences that have been previously associated with marine environments (indicated by * in Figures 2 & 3 and references provided in 9, 3-13); these include sediments but not strains typically found on water columns. The authors are careful in suggesting that bioaerosols derived from beaches and/or coastal erosion processes might be of potential importance.

27. The discussion appears lengthy and could be written in a more concise manner..For instance, I suggest deleting 11, 3-10

Response: Discussion will be re-worked.

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I think it would be appropriate to cite the following study, which to me appears very relevant to the present study: Camilla Fahlgren, Åke Hagström, Douglas Nilsson, and Ulla Li Zweifel Annual Variations in the Diversity, Viability, and Origin of Airborne Bacteria *Appl. Envir. Microbiol.*, May 1, 2010; 76: 3015 - 3025 .

Response: The study mentioned above will be cited. The detection of *Pseudomonas*, *Xanthomonas*, and *Sphingomonas* genera in their data makes for an interesting and intriguing comparison. The following study will also be cited, this is a phylogenetic study conducted during a dust arrival and shows a data-set more similar to ours: Teruya Maki et al. Phylogenetic analysis of atmospheric halotolerant bacterial communities at high altitude in an Asian dust (KOSA) arrival region, Suzu City. *Science of The Total Environment*, Volume 408, Issue 20, 15 September 2010, Pages 4556-4562.

Figures 2 and 3. Accession numbers for GenBank sequences should be given in the trees. It is very unclear what the designations mean: A, DF, C, D, D7(H), B/C+, B – please, describe this clearly somewhere in the ms.

Response: Sequence designation will be clarified in the revised manuscript.

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